THE UNIVERSITY OF ALBERTA

STUDIES OF CHRONIC CEREBRAL VASOSPASM AFTER SUBARACHNOID HEMORRHAGE IN PRIMATES

BY

Michael Gerrik Nosko

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL 1986

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-32458-9

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Michael Gerrik Nosko

TITLE OF THESIS: Studies of Chronic Cerebral Vasospasm After Subarachnoid Hemorrhage in Primates

DEGREE: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1986

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

Michael Nosko M.D.
Division of Neurosurgery
8440 112 Street
2D3.74 WMC
University of Alberta
Edmonton, Alberta
T6G 2B7

June 20, 1986

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled STUDIES OF CHRONIC CEREBRAL VASOSPASM AFTER SUBARACHNOID HEMORRHAGE IN PRIMATES submitted by Michael Gerrik Nosko in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Experimental Surgery.

.....
B.K.A. Weir, M.D., supervisor

.....
D.A. Cook, M.A., D.Phil.

.....
T.R. Overton, Ph.D.

.....
D.P.J. Boisvert, M.D., Ph.D.

.....
M. Grace, Ph.D.

.....
R. Spetzler, M.D.
External examiner

This thesis is dedicated
to my wife, Deborah, and to
my parents, Joseph and June

ABSTRACT

Cerebral vasospasm is a complication which may follow a subarachnoid hemorrhage. The resulting morbidity and mortality may approach 30% in those patients suffering from rupture of an aneurysm.

These studies were designed to characterize further the primate model of chronic cerebral vasospasm developed in this laboratory and to evaluate two modalities of treatment: use of the calcium antagonist, nimodipine, and early surgery during which an attempt is made to effect complete evacuation of the subarachnoid hematoma.

Study A

Using microsurgical procedures, an autologous hematoma was placed directly against the major anterior cerebral vessels in the right basal subarachnoid spaces of 24 cynomolgus monkeys. The monkeys were distributed at random to one of 4 groups and were treated orally q8h for 7 days with nimodipine (3, 6, or 12 mg/kg) or with placebo. Two additional monkeys underwent the surgical procedure without clot placement (sham-operated animals). Indices monitored before and after induction of the subarachnoid hemorrhage included: neurological status, angiographic cerebral vessel caliber and cerebral blood flow.

Significant vasospasm was present on day 7 in 83% of the animals ($p < 0.001$). There was no significant difference in the incidence of vasospasm or reduction of mean vessel caliber among the 4 groups. Sham-operated animals had no vasospasm on day 7. Two animals in the

group receiving the highest dose of nimodipine suffered delayed ischemic deficits.

High dose oral nimodipine is not effective in reducing the incidence of chronic cerebral vasospasm, or delayed ischemic deficits, after subarachnoid hemorrhage in the primate model.

Animals were assigned at random prior to sacrifice to either pathology or pharmacology subgroups. The pathology subgroup underwent *in vivo* cerebral fixation and the cerebral vessels were examined using electron microscopy. There were no differences in the pathological changes between treatment groups. The middle cerebral arteries of animals in the pharmacology subgroup underwent *in vitro* evaluation of contractility to norepinephrine, 5-hydroxytryptamine, and potassium chloride. There was a significant reduction in the response of the clot-side middle cerebral artery relative to the non-clot-side to all three agonists. This was not influenced by nimodipine treatment at any of the four doses tested. The non-clot arteries of the 12 mg/kg treatment group demonstrated enhanced reactivity to the agonists.

In vitro evaluation and comparison of dose-response curves of normal cerebral arteries from monkey, dog and human to norepinephrine, potassium chloride, prostaglandin $F_{2\alpha}$, 5-hydroxytryptamine and hemoglobin, with and without nimodipine blockade, revealed that monkey vessels are less sensitive to nimodipine with respect to norepinephrine and 5-hydroxytryptamine. The responses to prostaglandin $F_{2\alpha}$ or hemoglobin were not significantly antagonized by nimodipine at any concentration tested.

In vivo infusions of nimodipine, (2 and 5 μg/kg/min), significantly enhanced cerebral blood flow in the cynomolgus monkey.

Study B

Sixteen monkeys underwent induction of bilateral subarachnoid hemorrhage. The hemorrhage was extended from the previous study to include the posterior circulation. Eight additional animals underwent sham procedures. At 24 hours post-hemorrhage, 8 monkeys in the clot-placement group underwent complete removal of the subarachnoid clot (clot-removal group). On day 7, significant vasospasm was present in all untreated animals. No significant vasospasm was evident in the sham or the clot-removal groups. Two untreated animals developed delayed ischemic deficits documented by magnetic resonance imaging and pathology.

Removal of the hematoma 24 hours after a subarachnoid hemorrhage significantly decreases the incidence of chronic vasospasm in the primate model.

In vitro radioimmunoassay analysis of the basal levels of thromboxane A_2 and prostaglandin $F_{2\alpha}$ as their stable metabolites thromboxane B_2 and 6-keto-prostaglandin $F_{2\alpha}$ in the cerebral vessels of these monkeys revealed no differences in thromboxane B_2 levels among the groups. The 6-keto-prostaglandin $F_{2\alpha}$ levels of the clot group were significantly lower than the clot-removal group ($p < 0.01$).

Decreased prostacyclin production within cerebral arteries may contribute to the etiology of chronic vasospasm after subarachnoid hemorrhage in the monkey.

Key Words - primate; subarachnoid hemorrhage; nimodipine; pharmacology; cerebral blood flow; early surgery; clot evacuation, prostacyclin.

PREFACE

The onset of vasospasm brings with it a poor prognosis for the patient who has survived the initial insult of an aneurysm rupture. In spite of extensive research, the successful treatment and prophylaxis of vasospasm has remained an elusive goal. Progress has been hindered by the lack of an animal model which closely mimics the clinical experience with subarachnoid hemorrhage.

The last twelve years in the Cerebrovascular Research Laboratory at the University of Alberta have seen a steady progression towards the development of a reliable, reproducible primate model of the chronic cerebral vasospasm which often follows a subarachnoid hemorrhage. The work of each of the successive research fellows has served as a building block for the research of the next. Over the last four years, the primate model has developed to the stage at which vasospasm can be consistently produced in 90% of the animals in which a subarachnoid hemorrhage is induced. More recently, a 25% incidence of delayed ischemic deficits resulting from vasospasm has been achieved. This exciting advance promises the ability to conduct more definitive studies into the etiology, prevention, and therapy of chronic vasospasm and delayed ischemic deficits than have previously been possible.

The first part of the work presented in this thesis is directed at furthering the study done by Dr. F. Espinosa on the effect of the calcium blocker nimodipine in preventing vasospasm. The second part

looks at the question of the role of attempted complete, early removal of the clotted blood after a subarachnoid hemorrhage.

It is hoped that these studies will serve as a stepping stone for future fellows in the laboratory and that the model will continue to be developed and refined so that proposed new therapies will have a forum for rigorous evaluation. Continued research in this vein will eventually relegate vasospasm to a place in the past.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my appreciation to Dr. Bryce Weir for having given me the opportunity to work in the cerebrovascular laboratory and for his invaluable guidance.

I would like to thank Dr. David Cook for having provided me with his expertise and facilities for the pharmacological aspects of the research.

I also thank Dr. Thomas Overton for his timely criticisms and for providing the laboratory facilities; Dr. Michael Grace for our many discussions on statistical analysis; Dr. Donald Boisvert for his welcomed advice; and Dr. Bruce Mielke for reviewing the pathology.

I would also like to thank the staff of the Nuclear Magnetic Resonance facility, in particular Dr. P. Allen and Dr. A. Lunt.

The radioimmunoassays in Study B(*ii*) were performed by R. Schulz.

This work was in a large part possible only because of the excellent technical assistance of C. Krueger, T. Gorodetski, and L. Russell, as well as the staff of the Department of Applied Sciences in Medicine: C. Preshing, N. Ouellette, E. Haska, and R. Heath.

I would like to thank G. Hawkins for the special attention he provided to the animals in the vivarium and for his help on many occasions.

I am indebted to my wife, Debbie, for the many hours that she spent typing from barely legible drafts in order to produce this thesis.

Finally, I would like to mention Dr. A. Scriabine of Miles Laboratories for providing the nimodipine used in the study.

This study was supported by grants from the Medical Research Council of Canada, The Canadian Heart Foundation, and Miles Laboratories.

I am grateful to the Alberta Heritage Foundation for Medical Research for providing me with a Fellowship for the duration of this research.

TABLE OF CONTENTS

Page

Chapter One: Cerebral Vasospasm

I. An Overview 1

II. Etiology of Vasospasm 4

III. Prophylaxis and Treatment of Cerebral Vasospasm 14

IV. Calcium Entry Blockers 17

V. Clot Removal 23

VI. Summary 28

Chapter Two: The Present Studies

I. Objectives and Experimental Design 29

Study A: Nimodipine and Chronic Vasospasm
Objectives 29
Design 30

Study B: Clot Removal
Objectives 33
Design 33

II. Materials and Methods:

Animal Preparation 35

Subarachnoid Hemorrhage Induction 37

Clot Removal 40

Cerebral Angiography 40

Cerebral Blood Flow 42

Drug Administration 42

Neurological Assessment 43

Computed Tomography and Magnetic Resonance Imaging 44

Page

Pathology 45

Pharmacology 47

Data Analysis 50

Chapter Three: Results

Study A: Nimodipine and Chronic Vasospasm

Study A(*i*): Clinical, Radiological and Pathological Findings 52

Cerebral Vasospasm 54

Cerebral Blood Flow 63

Neurological Assessment 63

Nimodipine Levels 68

Pathology 68

Study A(*ii*): Pharmacological Studies of Vessels in Spasm 68

Study A(*iii*): *In Vitro* Effect of Nimodipine 78

Study A(*iv*): Intravenous Nimodipine and Cerebral Blood Flow 84

Study B: Early Clot Removal and Cerebral Vasospasm

Study B(*i*): Clinical, Radiological and Pathological Findings 91

Cerebral Vasospasm 93

Imaging 99

Pathology 105

Study B(*ii*): Basal Prostacyclin and Thromboxane A_2 Production 105

Chapter Four: Discussion 110

Page

Chapter Five: Conclusions and Recommendations 126

Bibliography 129

Publications 148

LIST OF TABLES

Table		Page
I.	Nimodipine and Chronic Cerebral Vasospasm	53
II.	Degree of Vasospasm Developed By Treatment Group: Study A(*i*)	55
III.	Development of Collateral Circulation By Treatment Group	64
IV.	Serum Nimodipine Assays By High Performance Liquid Chromatography	69
V.	Clot Removal and Chronic Cerebral Vasospasm	92
VI.	Degree of Vasospasm Developed By Treatment Group: Study B(*i*)	95

LIST OF FIGURES

Figure		Page
1.	Induction of Subarachnoid Hemorrhage	39
2.	Cerebral Angiograms of Sham Group Animal	56
3.	Cerebral Angiograms of Placebo Group Animal	57
4.	Cerebral Angiograms of Nimodipine 3 mg/kg Group Animal	58
5.	Cerebral Angiograms of Nimodipine 6 mg/kg Group Animal	59
6.	Cerebral Angiograms of Nimodipine 12 mg/kg Group Animal	60
7.	Grouped Average Constriction of Major Cerebral Vessels	61
8.	Grouped Mean Percentage Constriction of Major Cerebral Vessels By Treatment Group	62
9.	Cerebral Angiograms of Monkey No. 36	65
10.	Percentage Constriction of Major Cerebral Vessels of Monkey No. 36	66
11.	Pathology of Cerebral Infarct of Monkey No. 36	67
12.	Scanning Electron Micrographs of Middle Cerebral Arteries	70
13.	Scanning Electron Micrographs of Middle Cerebral Arteries	71
14.	Transmission Electron Micrographs of Middle Cerebral Arteries	72
15.	Scanning Electron Micrographs of Non-spastic Vessels	73
16.	*In Vitro* Response of Placebo Group Middle Cerebral Artery Rings	74
17.	*In Vitro* Response of Nimodipine 3 mg/kg Group Middle Cerebral Artery Rings	75

Figure Page

18. *In Vitro* Response of Nimodipine 6 mg/kg Group Middle Cerebral Artery Rings 76

19. *In Vitro* Response of Nimodipine 12 mg/kg Group Middle Cerebral Artery Rings 77

20. *In Vitro* Response of Isolated Middle Cerebral Artery Rings: Clot Side 79

21. *In Vitro* Response of Isolated Middle Cerebral Artery Rings: Non-Clot Side 80

22. *In Vitro* Response of Isolated Middle Cerebral Artery Rings: Monkey No. 36 81

23. *In Vitro* Effect of Nimodipine on Potassium Chloride Induced Contraction 82

24. *In Vitro* Effect of Nimodipine on 5-Hydroxytryptamine Induced Contraction 83

25. *In Vitro* Effect of Nimodipine on Norepinephrine Induced Contraction 85

26. *In Vitro* Effect of Nimodipine on Prostaglandin $F_{2\alpha}$ Induced Contraction 86

27. *In Vitro* Effect of Nimodipine on Hemoglobin Induced Contraction 87

28. Cerebral Blood Flow Response to Intravenous Nimodipine 2 μg/kg/min 88

29. Cerebral Blood Flow Response to Intravenous Nimodipine 5 μg/kg/min 90

30. Cerebral Angiograms of Sham Group Animal 94

31 Cerebral Angiograms of Clot Group Animal 96

32. Cerebral Angiograms of Clot-Removal Group Animal 97

33. Mean Percentage Constriction of Major Cerebral Vessels By Treatment Group 98

34. Magnetic Resonance Images of Clot Removal 100

35. Magnetic Resonance Images and Pathology of Monkey No. 100 102

Figure

Page

36. Magnetic Resonance Images and Pathology of Monkey No. 105 103

37. Pathology of Monkey No. 125 106

38. *In Vitro* Evaluation of Basal Levels of 6-Keto-Prostaglandin $F_{1\alpha}$ 107

39. *In Vitro* Evaluation of Basal Levels of Thromboxane B_2 108

CHAPTER ONE: CEREBRAL VASOSPASM

AN OVERVIEW

Cerebral vasospasm is a term which has been used to describe an angiographic and clinical condition which may follow a subarachnoid hemorrhage. While it has been described as occurring after head injury (107, 146, 213), ruptured arteriovenous malformation (151), hypothalamic-pituitary surgery (24, 110), and intracranial infection (104), it most commonly follows a subarachnoid hemorrhage due to rupture of a cerebral aneurysm (30).

Chronic cerebral vasospasm may be defined as a reversible exaggerated constriction of the cerebral vessels in response to perivascular blood in the subarachnoid space. After the rupture of a cerebral aneurysm, focal or diffuse narrowing of one or more intracranial arteries, proximal or distal to the site of rupture, may be seen by angiography. This was first described by Ecker et al. in 1951 (34). The spasm may be mild, moderate, or severe, and, notwithstanding any of the above, may or may not be symptomatic.

Vasospasm usually develops between days 4 and 12 after the first subarachnoid hemorrhage and the peak incidence is between days 6 and 8; it then generally resolves over 1 to 2 weeks, although it can persist for longer periods (32, 108, 146, 200, 204).

Approximately 76% of all primary subarachnoid hemorrhages result from ruptured aneurysms (129). The incidence of aneurysmal subarachnoid hemorrhage varies from 3.5/100,000 population to a high of 25/100,000 population (129, 136, 163).

In Canada, approximately 3,000 new cases of subarachnoid hemorrhage due to aneurysm rupture present each year (12/100,000 population)(33, 85). A similar incidence in the United States results in 28,000 new cases per year (82).

The mortality rate from aneurysmal subarachnoid hemorrhage is high. Within the first 24 hours after hemorrhage, between 8% and 20% of patients die. At 48 hours, mortality rises to 20-25%; at 14 days, 44-56%; and at 2 months, 66% (85, 102, 136, 169, 203, 208).

Approximately one third to one half of the 60% of patients who survive the initial ictus will experience a deterioration in neurological status in the two weeks following the subarachnoid hemorrhage (45). This represents about 10,000 patients across North America. While the principal causes of this delayed morbidity and mortality include vasospasm, re-bleeding, hydrocephalus, and post-operative complications, the most significant cause is chronic cerebral vasospasm (74, 82, 141, 202). While some have questioned the significance of angiographic vasospasm (142), it is generally agreed that the narrowing in arterial diameter can be sufficiently severe as to result in focal cerebral ischemia and infarction (30, 46). After rupture of an aneurysm, symptomatic vasospasm occurs in 25-37% of patients. Permanent neurological deficit or death occurs in 7-17% (7, 62).

Decreased cerebral blood flow, neurological status post-hemorrhage, delayed neurological deficits, general prognosis, and pathological findings have all been positively correlated with angiographic findings of severe symptomatic vasospasms (1, 63, 66, 74, 113, 137, 141, 158, 201, 202). One study demonstrated that 85%

of cerebral hemispheres with vasospasm had ischemic damage in the distribution of the affected vessels, showing a significant relationship between angiographic vasospasm and delayed ischemic deficits (64). However, such infarctions do not invariably accompany angiographic reduction in vessel caliber (30).

The evaluation of the size of the subarachnoid hematoma as seen on CT scans, shortly after the hemorrhage, appears to be the most reliable method of predicting the occurrence and severity of vasospasm (88, 161, 215). In a prospective study, 20 of 22 patients who demonstrated thick hematomas in the subarachnoid space after an aneurysm rupture, developed severe vasospasm. Only 5 of 19 patients showing little or no subarachnoid blood developed severe vasospasm. It was thus concluded that the development of severe vasospasm could be predicted with a reasonable degree of accuracy (88). Another study confirmed these data, reporting that the presence or absence of vasospasm positively correlated with the quantity and distribution of subarachnoid blood as seen on CT scan (161).

The development of delayed ischemic deficits due to vasospasm is a phenomenon which occurs over a period of time. The onset involves a reduction in consciousness, mild pyrexia (38° to 39°C), followed by signs of ischemia in the territory of the spastic artery. While the onset may occur within 4 to 5 days of the hemorrhage, the deficit tends to progress until it is completed 2 to 4 days after onset. It may, however, peak as early as 24 hours (45). Approximately 50% of the deficits are potentially reversible, or of only a transitory nature. Progression of the ischemia can lead to single or multifocal infarcts or massive infarction causing mild to severe neurological

debilitation or, in extreme cases, causing death (15, 46, 66, 82, 113, 143, 146, 201, 202). Heros et al. performed a study of 86 patients with ruptured aneurysms. Eleven patients who died pre-operatively demonstrated severe vasospasm and pathologically proven ischemic infarction. Post-operative vasospastic ischemia developed in 14 patients, of whom 4 died. In their experience, the incidence of vasospasm was not affected by size or location of aneurysm, age, hypertension, arteriosclerosis, or intraoperative hypotension (73).

Fisher et al. determined that in patients who had suffered symptomatic or severe vasospasm, the angiographic vessel caliber returned to normal in 70% within 28 days (46).

Etiology of Vasospasm

Many agents have been purported to play a role in the genesis of vasospasm. These include, but are not limited to: whole blood, erythrocyte breakdown products, hemoglobin (112, 119, 127, 185, 187, 220), fibrin degradation products (52, 53, 103), prostaglandins (18, 25, 149, 191), thromboxane A_2, 5-hydroxytryptamine (159, 174, 175, 195), potassium, catecholamines (98, 159, 174), histamine (99), vasopressin and angiotensin (60). It has also been suggested that vasospasm is a multifactorial phenomenon (188).

Svendgaard et al. produced experimental subarachnoid hemorrhage in rabbits by injecting autologous blood intracisternally. They found that at 3 days after the hemorrhage, the basilar artery of the animals showed an increased reactivity to norepinephrine and 5-hydroxytryptamine relative to untreated control animals (174). A

similar series of studies in cats by Lobato et al. demonstrated a dramatic supersensitivity to norepinephrine and 5-hydroxytryptamine that peaked 3 days following the intracisternal injection of blood, and gradually disappeared over 30 days.

Similar effects have been observed after ganglionectomy or treatment with 6-hydroxydopamine. These authors suggested that vasospasm may result from a supersensitivity of the cerebral arteries to catecholamines (100). The majority of these studies, however, fail to demonstrate the existence of vasospasm in the experimental animals. Toda et al. (186) obtained an opposing result. Using a dog model, subarachnoid hemorrhage was induced by removing a previously placed needle which transfixed the internal carotid-posterior communicating arteries. Angiographic evidence of vasospasm was obtained at various time intervals after the induction of the hemorrhage. *In vitro* analysis of the contractility of middle cerebral artery strips led the authors to conclude that the artery on the side of the induced hemorrhage was much less sensitive to all of the agonists tested and that supersensitivity was, therefore, not the primary mechanism of vasospasm secondary to subarachnoid hemorrhage. They also observed that the arterial contractile response was much less for norepinephrine than to other agents, including 5-hydroxytryptamine, histamine, and potassium chloride.

Cerebrospinal fluid from patients who have suffered an aneurysmal subarachnoid hemorrhage is known to cause vasoconstriction of isolated cerebral vessels. Studies which have investigated the contractile properties of hemorrhagic cerebrospinal fluid have failed to conclusively identify the vasogenic substance or substances, but

5-hydroxytryptamine, histamine, norepinephrine, epinephrine, acetylcholine, angiotensin II and potassium were ruled out as possible causes of chronic vasospasm (19, 126). Similarly, a study which evaluated the concentration of 5-hydroxytryptamine in cerebrospinal fluid from subarachnoid hemorrhage patients and controls could find no correlation between the cerebrospinal fluid 5-hydroxytryptamine concentration and the clinical grade of subarachnoid hemorrhage, or the severity or incidence of vasospasm. Clinical studies evaluating the efficacy of the 5-hydroxytryptamine antagonists reserpine and kanamycin have also failed to demonstrate any beneficial effect of suppressing serum 5-hydroxytryptamine levels on the development of vasospasm (16, 195). Further studies by Sasaki et al. (195) supported the exclusion of 5-hydroxytryptamine, norepinephrine, acetylcholine and histamine from playing a role in the production of vasospasm. They determined that the vasoconstrictor activity of hemorrhagic cerebrospinal fluid was not reduced by the addition of neurotransmitter antagonists methysergide, mepyramine, phenoxybenzamine, propranolol, or atropine. However, the activity was significantly reduced by the reducing agents dithiothreital and dithioerythrital which reduce disulfide bonds, such as those contained in prostaglandin receptors (54). Furthermore, the antagonism of the vasospastic activity was reversed by the oxidizing agent 5-5'-dithiobis-2-nitrobenzoic acid. These findings led the authors to speculate that prostaglandins, hemoglobin, and lipid hydroperoxides may be the vasospastic agents in hemorrhagic cerebrospinal fluid.

Xanthochromia has been demonstrated within 4 hours of a subarachnoid hemorrhage (109). In general, subarachnoid clot lysis begins within 24 hours of the hemorrhage and peaks around 5 to 7 days post-hemorrhage (91, 153). Hemoglobin is known to be a vasoconstrictor, but pure hemoglobin is not as potent as its hemolysate (69). Studies of intact and lysed erythrocytes have shown that lysed erythrocytes are more vasospastic than intact cells and that the vasogenic activity is directly correlated to the concentration of oxyhemoglobin (10, 81, 124, 125, 146).

It has been suggested that a combination of oxyhemoglobin, lipid peroxides and prostaglandins may be the cause of chronic vasospasm (146). While oxyhemoglobin itself is a potent vasoconstrictor (164), it has been shown that oxyhemoglobin is rapidly oxidized to methemoglobin in the subarachnoid space. Interestingly, the spasmogenic activity of oxyhemoglobin can be reduced by the action of superoxide dismutase or catalase (80). The oxidation reaction of oxyhemoglobin produces superoxide radicals which peroxidize fatty acids in the cell membranes (10, 153, 154). Lipid hydroperoxides have been shown to cause a biphasic cerebral vasospasm which follows a similar time course to human chronic vasospasm (154).

While superoxide radicals do not appear to have any vasogenic activity of their own (207), the production of lipid hydroperoxides can cause myonecrosis of the vascular tunica media and can induce degeneration of the endothelium. The state of integrity of the endothelium is well known to have an effect on the *in vitro* reactivity of cerebral vessels (57). The arachidonic acid cascade takes place within the endothelial cells of the cerebral vasculature.

While many products of this system are synthesized in cerebral arteries, including prostaglandin $F_{2\alpha}$, $F_{1\alpha}$, D_2, and thromboxane A_2 (67), the predominant prostaglandin synthesized appeared to be prostacyclin (18, 106, 152). Prostacyclin is a potent inhibitor of cerebral vasoconstriction in concentrations of 10^{-10} to 10^{-6}M. At concentrations above 10^{-5}M, it acts as a vasoconstrictor itself. Prostacyclin abolishes the *in vitro* contractile response of prostaglandins F_2, D_2, E_2, H_2; thromboxane A_2; norepinephrine; 5-hydroxytryptamine; and angiotensin (9, 20, 22, 23, 55, 130, 191, 196, 216). Upon damage to the vascular endothelium, as can occur with lipid peroxidation, the synthesis of prostacyclin is reduced while the synthesis of other products of the arachidonic acid cascade appear unaffected (22, 37, 77). *In vitro* exposure of canine cerebral vessels to blood for 3 to 8 days decreased the synthesis of prostacyclin from control levels, but had no effect on the synthesis of vasoconstrictor prostaglandins (152). *In vivo* experimental subarachnoid hemorrhage studies with canine cerebral arteries demonstrated a decrease of prostaglandin $F_{1\alpha}$ synthesis and an increase in prostaglandin E_2 synthesis on days 2 and 6 post-hemorrhage (105). Several studies have evaluated the concentration of vasoconstrictor prostaglandins in the cerebrospinal fluid of normal patients and patients who had suffered an aneurysmal subarachnoid hemorrhage. The general findings are in agreement that the cerebrospinal fluid concentration of prostaglandin $F_{2\alpha}$, E_2, and thromboxane B_2 are increased in the subarachnoid hemorrhage patients (56, 90, 197). In a recent study, cerebrospinal fluid levels of prostaglandin D_2 and 6-keto prostaglandin $F_{2\alpha}$ were monitored with

serial lumbar punctures. In patients with radiographic vasospasm, prostaglandin D_2 concentrations paralleled the extent of the vasospasm, whereas prostacyclin synthesis appeared inhibited. When there was no evidence of vasospasm, arachidonate metabolite concentrations appeared stable (12).

In vitro studies, using human cerebral arteries which had been obtained within 10 hours of death, evaluated the effects of leukotrienes C_4 and D_4, prostacyclin, and thromboxane A_2 (196). The leukotrienes had no vasoconstricting or vasodilating effect on the human basilar artery strips. Thromboxane A_2 had a powerful vasoconstriction effect in a dose-dependent manner at concentrations of 0.03 nM to 0.15 nM. The prostacyclin effected a dose-dependent relaxation at concentrations of 0.2 nM to 0.8 nM. Furthermore, prostacyclin had an inhibiting effect when given concurrently with a vasospastic substance. An *in vivo* study in cats evaluated intravenous infusion of prostacyclin into animals in which prolonged cerebral vasospasm was induced by continuous application of an oxyhemoglobin solution around the basilar artery. Vasospasm was reversed in all animals at a dose of 50 μg/kg/min. No significant hypotension was produced at this dosage (139). Other studies have not corroborated these findings. Vertebral artery infusion in dogs 24 hours after hemorrhage failed to reverse the acute vasospasm. Similarly, intravenous infusion in cats (25-75 ng/kg/ml) 5 days after induction of subarachnoid hemorrhage failed to reverse the chronic vasospasm, but did cause significant hypotension (55, 211).

While a role for the clinical use of prostaglandin manipulators is still to be realized, the general belief is that prostaglandins,

and possibly thromboxanes, do play a role in the genesis of vasospasm. Free radical release upon the oxidation of oxyhemoglobin to methemoglobin after subarachnoid hemorrhage enhances the peroxidation of arachidonic and linoleic acids. Endoperoxide production is thus increased which appears to stimulate the synthesis of constrictor prostaglandins. Thromboxane A_2 is produced in abundance in platelets and since platelets aggregate to an area of damaged endothelium, this source may be sufficient to produce vasospasm. The vasodilator, prostacyclin, however, is produced by the vascular endothelium. Lipid peroxidation damages the endothelial cell walls, causes myonecrosis of the tunica media, and may severely inhibit prostacyclin synthesis. Therefore, vasospasm conceivably results from the combined action of oxyhemoglobin, lipid peroxides, an increase in vasospastic prostaglandins and a decrease in vasolytic prostacyclin.

The final common pathway of all vasospastic substances is the contraction of the smooth muscle cells of the tunica media. These cells have receptors for a wide variety of agonists. There are at least 5 known receptors for prostaglandins which appear to be separate from the adrenergic and cholinergic receptors as they are not blocked by propranolol, hexamethonium, phenoxybenzamine, mepyramine, methysergide, or atropine (75).

While the exact physiology of the events of membrane receptor coupling, transduction and activation of the contractile mechanism of smooth muscle is still unclear, an understanding of the basic interactions is required in order to have a basis for the rationale of treatment.

Muscular contraction, whether skeletal or smooth, is dependent upon intracellular calcium levels. Calcium is present in the body either as a free ion or bound to protein complexes. The serum concentration of free calcium is approximately 1.15×10^{-3}M. Total intracellular calcium levels depend on the type of muscle cell. Intracellular free calcium concentration is dependent upon the state of contraction of the muscle cell and varies from 1.8×10^{-7}M in a relaxed state to 1×10^{-5}M in a maximally contracted state (4, 206).

The process of smooth muscle contraction is set in motion by one of 2 mechanisms: stimulation of one of the many types of receptors on the smooth muscle cell membrane, or by depolarization of the membrane.

In order to initiate smooth muscle cell contraction, 4 molecules of free intracellular calcium must bind to 4-divalent binding sites on the protein calmodulin, which is a regulatory enzyme. This now activated calcium-calmodulin complex binds to the inactive myosin light chain kinase (MLCK) to form a catalytically active holoenzyme. Activated MLCK then acts as a catalyst to enable the phosphorylation of the myosin light-chain subunit. The phosphorylated light chain stimulates the actin-myosin interaction with free intracellular calcium forming bridging links between the two molecules. This gives rise to the constriction of the smooth muscle cell. The energy of constriction is provided by the dephosphorylation of adenosine triphosphate to adenosine diphosphate by the enzyme adenosine triphosphatase (ATP-ase). As the intracellular concentration of calcium increases, so does the contractile tension and the activity of ATP-ase which achieves a relatively steady state for calcium

transfer between the intracellular pool and the extracellular space. As calcium influx decreases, membrane Ca-ATP-ase removes cytoplasmic calcium and as the concentration of free intracellular calcium decreases, the myosin P light chain is dephosphorylated and the contractile apparatus disengages (162, 192, 209). Thus the degree of contraction is controlled by regulation of the concentration of free intracellular calcium. Calcium is extruded from the cell by several transport systems. The most active appears to be a Ca-ATP-ase which actively transports free calcium across the cell membrane. The control over free intracellular calcium levels seems to be affected by cyclic adenosine monophosphate and cyclic guanine monophosphate. The conversion of adenosine triphosphate to cyclic adenosine monophosphate decreases free calcium and increases protein based calcium, ultimately stimulating relaxation of the smooth muscle. The conversion of guanine triphosphate to cyclic guanine monophosphate antagonizes this reaction (89, 209). Cyclic adenosine monophosphate phosphodiesterase inhibitors such as aminophylline and papaverine are capable of decreasing smooth muscle tone. Similarly, dibutyryl cyclic adenosine monophosphate can also decrease smooth muscle tone. Cyclic guanine monophosphate levels are decreased by the vasodilators sodium nitroprusside and nitroglycerine. These data provide support for cyclic adenosine monophosphate and cyclic guanine monophosphate being the secondary messengers for receptor stimulated relaxation and contraction respectively (3, 47, 134).

There are two sources of free intracellular calcium. It can be released from the bound intracellular stores, or it can influx from the extracellular pool. The phospholipid bilayer cell membrane of

the smooth muscle cell separates the intracellular pools from the extracellular space. Within this membrane are channels through which ions may pass bidirectionally. There are at least two types of influx calcium channels in smooth muscle cells: potential dependent channels; which are opened by membrane depolarization, and receptor operated channels which are opened when the appropriate receptor is activated. This two channel system has been demonstrated by selectively blocking potential dependent channels with verapamil and thus preventing contraction due to high extracellular potassium concentrations. Such cells are still capable of contraction upon exposure to norepinephrine which stimulates receptor operated channels. There is a third pathway for calcium to gain access to a cell, and that is by passive leakage. This continuous leakage is resistant to blockade and is present at all times including when the cell is in the resting state and potential dependent channels and receptor operated channels are closed (14, 192). It is probably this continual leakage which is responsible for the resting tone of the smooth muscle cell. Two types of intrinsic smooth muscle tone have been found. The first is a rhythmic pulse which is dependent upon calcium induced action potentials and thus is sensitive to calcium channel blockade. The second tone is a constantly maintained tone independent of calcium channel action potentials. This maintained tone is independent of innervation, prostaglandins and angiotensin, but is dependent on extracellular calcium concentrations. It is for these reasons that intrinsic maintained tone is believed to be sustained by the passive intracellular calcium leakage.

Contractions due to membrane depolarization are highly dependent on extracellular calcium and therefore are relatively easy to block. Receptor activated contraction is less dependent on extracellular calcium in that it appears to proceed when all extracellular calcium has been removed. Receptor activated contractions must therefore be able to draw on intracellular calcium stores. The main storage site for bound intracellular calcium is the sarcoplasmic reticulum and, to a lesser extent, the plasma membrane. Mitochondria also contain large stores of calcium, but whether this site is physiologically important for cellular contractions has been argued (165, 166).

Smooth muscle cell contraction has been found to be partially affected by ambient temperatures. Muscle tone increases as the temperature increases from 34°C. At 42°C, muscle contraction becomes independent of extracellular calcium. Since subarachnoid hemorrhage has been associated with pyrexia, it is conceivable that the fever may contribute to post-hemorrhage vasospasm (14, 143, 209).

Prophylaxis and Treatment of Cerebral Vasospasm

The optimal prophylaxis of cerebral vasospasm is the prevention of the aneurysmal subarachnoid hemorrhage which causes it. At present, there is no diagnostic tool which is sufficiently sensitive, specific, or cost/benefit effective for the routine screening of the general population. Cerebral pan-angiography is at present the only modality which reliably determines the presence of a pre-rupture aneurysm. This technology is too costly in both a morbid and a

financial sense to screen even a limited number of the at-risk general population.

The focus must therefore shift to the prevention of vasospasm once the subarachnoid hemorrhage has occurred. A review of agents which have been used in an attempt to prevent, or to relieve, established cerebral vasospasm revealed that over 80 different regimes have been tried, and none has been consistently successful (212).

Attempts to suppress the activity of supposed vasoconstrictor nerves with high dose hydrocortisone initially showed some promise, but clinical studies were inconsistent (72). Combined therapies using a variety of alpha and beta blockers and agonists such as phenylephrine with sodium nitroprusside, or phentolamine with propranolol, have also met with varying success (5, 198).

Studies have been designed in attempts to lower the cerebral and plasma levels of circulating monoamines. Reserpine and kanamycin received much attention in this regard. Zervas observed that treatment with these agents could prevent vasospasm in dog and monkey models of subarachnoid hemorrhage (219). Clinical trials have been less encouraging (15).

The discovery of the potent cerebral vasodilator prostacyclin and the vasoconstrictor thromboxane A_2 has led to research to find ways of promoting and inhibiting their actions. Prostacyclin predictably dilates cerebral vessels *in vitro* and *in vivo*, but its short half-life hinders clinical trials. Inhibition of thromboxane A_2 synthesis may also have a role in vasolytic therapy (118, 176). Pyridine and imidazole have been shown to inhibit thromboxane A_2

production. More recently a pyridine derivative Sodium- (E) -3- (4 (3-pyridylmethyl) phenyl) -2-methyl-2-propenoate (OKY-1581) was found effective in preventing experimental vasospasm (155).

The development of hyponatremia and hypovolemia which often precedes the clinical vasospasm syndrome (74) has led to the suggestion of hypervolemia and arterial hypertension as therapeutic agents (43, 84, 214). The principle involved is to attempt to maintain cerebral perfusion pressure in spite of significant cerebral arterial constriction. Expansion of the intravascular volume may be accomplished with whole blood, packed cells, albumin, or low molecular-weight dextran. Vasopressin and fludrocortisone may assist in maintaining the hypervolemic state. Dopamine and atropine maintain hypertension. In the presence of disturbed cerebral autoregulation, this therapy may considerably affect cerebral edema. Pulmonary edema has also been a complication of this treatment modality. The most serious drawback is the risk of re-bleeding of an unrepaired ruptured aneurysm. Aggressive therapy of this nature must be restricted to patients who have already undergone successful repair of the aneurysm.

One of the most promising groups of therapeutic agents to be investigated recently is the calcium antagonist. If cerebral vasospasm is initiated, and possibly maintained, by the sustained contraction of the medial smooth muscle layer, then precluding the contraction of the vascular smooth muscle cell should prevent the development of the vasospasm regardless of its primary etiology. Several potential methods for this type of intervention have been summarized by Towart (188).

Calcium Entry Blockers

The calcium entry blockers are a chemically heterogenous family of compounds that have been available commercially for the last 20 years (49, 199). Various subgroups operate at predominantly different sites. The phenothiazine antipsychotic agents function at the myofilament level by blocking calcium-calmodulin interactions, thus inhibiting the contractile process. Another group, the dihydropyridines, appears to inhibit calcium influx through the smooth muscle cell membrane, thereby preventing the initiation of an increase in intracellular free calcium required for contraction to occur. This discussion will focus on this second class of calcium entry blockers.

There are presently three classes of calcium channels recognized in the smooth muscle cell membrane (14): potential dependent channels which are responsive to changes in the potential difference across the cell membrane; receptor operated channels which open in response to stimulation of a receptor by some agonist; and stretch dependent channels which are responsible for the slow inward calcium leak and the maintenance of tone.

Depolarization induced contraction of smooth muscle cells can be inhibited by calcium entry blockers. Calcium antagonists added to the bath of potassium depolarized coronary artery strips were able to inhibit the contractions. It has been shown that depolarization contractility is proportional to the extracellular calcium concentration (50, 51). Potassium depolarization induced contraction is readily inhibited by low doses of most of the calcium blockers (205). Towart et al. studied the effect of nimodipine on isolated

strips of rabbit aorta (189). Nimodipine was able to block potassium induced contractions, but not those induced by norepinephrine. The reverse was noted when the alpha-blocker phentolamine was used. The results of these studies indicate that the dihydropyridine calcium blockers inhibit the influx of calcium through potential dependent channels, but do not inhibit intracellular calcium pools. Receptor stimulation by norepinephrine probably releases stored intracellular calcium to initiate contractions. Similar results have been obtained with 5-hydroxytryptamine induced contractions (177).

Studies of nimodipine action on different vascular specimens have yielded varying results. Contractions induced by agonists such as 5-hydroxytryptamine, histamine, catecholamine, thromboxane and blood constituents were more potently inhibited on basilar arteries than on saphenous arteries (188). Varhoutte demonstrated similar findings with the calcium antagonist phenarizine on basilar versus gastrosplenic, coronary, or tibial arteries (193). Allen et al. have suggested that cerebral arterial smooth muscle is solely dependent on extracellular calcium for contractions induced by alpha-adrenergic, 5-hydroxytryptamine, or prostaglandin $F_{2\alpha}$ receptor stimulation intracellular calcium (8).

Allen suggested that cerebral arterial smooth muscle cells may have a greater dependence on extracellular calcium supplies than other types of smooth muscle (6). Others have come to similar conclusions (111, 121). This would render cerebral arterial contraction more susceptible to interference by calcium antagonists.

Studies supporting the cerebrovascular specificity of these calcium blockers have been numerous. Pearce and Benson found that *in*

vivo infusions of diltiazem, 30 μg/kg/min, dilated cerebral vasculature without affecting peripheral systemic resistance, cardiac output, mean arterial blood pressure, or cerebral metabolic rate (131). Verapamil, another calcium blocker, has been found to inhibit potential dependent contractions in all blood vessels tested, but it also inhibits receptor dependent contractions in cerebral arteries (48). Kazda and Towart found nimodipine to be effective in preventing 5-hydroxytryptamine induced contractions of rabbit basilar arteries but not systemic arteries (87).

Nifedipine and nimodipine have been found to be 10 and 6 times more effective, respectively, in preventing 5-hydroxytryptamine induced contractions of basilar over mesenteric arteries (120).

Nimodipine has also been found to inhibit basilar artery contraction to carbocyclic thromboxane, but not saphenous artery contraction (190). Thromboxane A_2 production has been found to be increased during periods of cerebral ischemia (59). Thromboxane A_2's powerful vasoconstrictor effect may set up a cycle of vasoconstriction/increased production. Nimodipine may be effective in inhibiting this cycle.

Many of the dihydropyridines increase cerebral blood flow. In dogs, intravenous injection of diltiazem, 0.1 mg/kg, increased cerebral blood flow 30%. Infusions of 30 μg/kg/min increased cerebral blood flow by 16%, decreased cerebrovascular resistance by 24% and had no effect on total peripheral resistance, mean arterial blood pressure, or cardiac output. Increasing the infusion to 100 μg/kg/min did decrease these latter variables (156). Intra-arterial injections of nimodipine, 0.01 mg/kg, into normal dogs increased

cerebral blood flow by 70% without affecting other hemodynamic variables (86). Nifedipine, 0.5 mg/kg, increased cerebral blood flow 33% in rats (145). Nimodipine causes a dose dependent increase in cerebral blood flow in the range 0.01 - 1.0 mg/kg while causing only a slight increase in mesenteric and renal blood flow (86). Primate studies have demonstrated that intravenous infusion of 2 μg/kg/min increased cerebral blood flow by up to 27% while decreasing mean arterial blood pressure up to 12%. Intracarotid infusions of 0.67 μg/kg/min increased cerebral blood flow by 57% in the normal animal and by 87% following hyperosmolar blood brain barrier disruption (70). Another primate study indicated that residual blood flow following occlusion of the middle cerebral artery was enhanced by nimodipine, but that at doses above 10 μg/kg/min, cerebral blood flow returned to control levels and mean arterial blood pressure fell. Furthermore, at the most effective dose for increasing cerebral blood flow, cerebrovascular responses to $PaCO_2$ and hypotension were reduced (71).

Cerebral blood flow was measured in 10 patients who had suffered an acute ischemic stroke. Patients were given nimodipine, 15 or 30 μg/kg, intravenously. All patients experienced a dose dependent increase in hemispheric cerebral blood flow. There was only one adverse reaction of hypotension and bradycardia which resolved spontaneously after a few minutes (61).

Oral doses of 40, 60, or 80 mg of nimodipine given to patients suffering an acute subarachnoid hemorrhage resulted in a mean increase in cerebral blood flow of 14% with no significant hemodynamic changes (58). Ott and Sechner showed that oral

nimodipine given to patients with cerebral infarction caused a redistribution of cerebral blood flow within the infarcted hemisphere with regional cerebral blood flow increasing in the infarcted area. There was no change in blood flow in the contralateral hemisphere (128).

The exact sites of action of nimodipine with respect to the cerebral blood flow have not been conclusively determined. Studies indicate however, that it is the smaller resistant arterioles which tend to be the most affected (181).

Topical nimodipine has also been evaluated clinically. Auer et al. applied a 2.4×10^{-5}M solution to the exposed cerebral vessels of patients operated on within 42 to 72 hours post-subarachnoid hemorrhage. In 13 patients, post-operative application was continued by means of a plastic cannula inserted intraoperatively (11). Vasodilation occurred in all patients and was relatively greater in the smaller vessels. Severe vasospasm was absent on day 7 post-subarachnoid hemorrhage in all cases. The authors concluded that topical application of nimodipine intraoperatively and post-operatively decreased the probability of symptomatic vasospasm after early surgery. The experimental evidence that nimodipine could be an effective treatment for cerebral vasospasm and delayed ischemic deficits led to a multi-center, prospective, randomized, double-blind placebo-controlled trial of the drug (7). One hundred and twenty-five neurologically normal patients were started on oral nimodipine (0.07 mg/kg loading dose; 0.35 mg/kg q4h for 21 days) or a placebo within 96 hours of having suffered a subarachnoid hemorrhage. A persistent deficit that was severe or caused death before the end

of treatment occurred in 8 of 60 patients given placebo but in only 1 of 56 given nimodipine. Since angiography was only repeated if a neurological deficit developed, the effect of nimodipine on the incidence and severity of vasospasm could not be ascertained. The results however, did provide strong evidence for a reduction in the incidence and severity of delayed neurologic deficits in good grade patients given oral nimodipine after subarachnoid hemorrhage.

Espinosa et al. studied the effect of oral nimodipine after subarachnoid hemorrhage in primates (41). In a randomized, placebo-controlled, blinded trial, 30 monkeys underwent subarachnoid hemorrhage induction and were then started on a 14-day regimen of nimodipine, 1 mg/kg q8h, or placebo. Significant vasospasm developed in 87% of the animals and its incidence was slightly higher in the placebo group. One monkey in the placebo group developed a delayed ischemic deficit which lasted until sacrifice. The effect of nimodipine on angiographic vessel caliber reduction at this dose was, however, not significant. Since there were no serious side effects, and no clear beneficial effects, it was suggested that higher doses of nimodipine be evaluated.

Recent work by Zabramski et al. (218) in a multi-hemorrhage dog model evaluated high dose oral nimodipine and nifedipine given for 7 days following subarachnoid hemorrhage induction. This group found no significant decrease in the severity of angiographic vasospasm at any of the doses tested.

A clinical study evaluated intracarotid slow bolus infusion of nimodipine (0.068 to 1 mg) given within 2 to 5 hours of angiographic demonstration of vasospasm in 6 subarachnoid hemorrhage patients

(65). Repeat angiography failed to show an immediate change in vessel caliber and the patients were started on constant intravenous infusions of nimodipine. Three patients died, 2 had major infarcts and 1 made a good recovery. The authors suggested that intracarotid infusion of nimodipine in cases of established vasospasm post-subarachnoid hemorrhage does not cause angiographic or clinical improvement.

Ljunggren et al. (95) also found no significant effect on the incidence and distribution of cerebral vasospasm following topical application (2.5×10^{-5}M) and intravenous infusion (2 mg/hr for 7 to 12 days) of nimodipine in 60 subarachnoid patients operated upon within 5 days of hemorrhage. Permanent delayed ischemic deficits occurred in 4% of patients as compared to 13% of a retrospective control group. The authors speculated that nimodipine must exert its anti-ischemic effects by some mechanism other than preventing or reversing angiographic vasospasm.

A more recent study by the same group (157) found that topical and intravenous administration of nimodipine did not prevent delayed ischemic deficits in patients operated upon early for an anterior communicating artery aneurysm. They did, however, find a decrease in permanent neurological sequelae in patients with a ruptured internal carotid or middle cerebral artery aneurysm.

Clot Removal

The suspected presence or radiographically demonstrated presence of cerebral vasospasm after subarachnoid hemorrhage is considered by many a contraindication for surgery to repair the offending aneurysm.

Surgery may place additional stress on cerebral tissue which has a compromised circulation. Delay in definitive treatment of a ruptured aneurysm increases the risk of a second and possibly fatal re-bleed. The optimal method of avoiding a secondary bleeding episode with its inherent increase in morbidity and mortality is to operate as early as possible after the initial insult.

The value of early surgery in the management of ruptured aneurysms has been stressed in several studies (31, 83, 96, 97, 115, 148, 172, 182, 215). A preliminary report on the findings of the cooperative study on the timing of aneurysm surgery also demonstrated an improved prognosis when ruptured aneurysms were operated upon quickly. While the risk of re-bleed with its subsequent morbidity and mortality is greatly reduced after definitive clipping of the aneurysm, the problem of chronic vasospasm is still present. The therapies previously discussed for established vasospasm are more safely applied when the source of the bleeding has been clipped. Cerebral perfusion pressures can be increased through the use of pressor agents and volume expansion, and increased intracerebral pressure can be treated without increasing the risk of a re-bleed from an unclipped aneurysm. The optimal method of treating cerebral vasospasm would be to prevent its initiation. As has been discussed, the etiology of vasospasm is presently unclear, but the general consensus is that it results from some components or by-products of the extravasated blood. Osaka suggested that if vasospasm is produced by the breakdown products of erythrocytes, then its prevention should be the complete removal of erythrocytes from the subarachnoid space (127). The possible role of mechanical removal of

blood from the basal cisterns in the prevention of chronic vasospasm was first suggested in 1958 by Johnson (78) and in 1959 by Pool (138).

Suzuki et al. (173) considered that removal of the clot from around the cerebral arteries in the basal cisterns within 24 hours of a subarachnoid hemorrhage greatly reduced the incidence of post-operative vasospasm in the 413 cases of hemorrhage from aneurysmal rupture that they studied. Takahashi et al. (179) demonstrated a positive correlation between the results of early operation performed within one week after the hemorrhage and CT findings. Symptomatic vasospasm developed in all cases in which the CT scans showed high density areas in the ventricles or cisterns. In cases with no blood clots evident within the ventricular system or the basal cisterns, those patients operated on within 24 hours of the hemorrhage had a 0% mortality and a 16% morbidity; those operated upon between days 2 and 7 post-hemorrhage exhibited a mortality rate of 38% and a morbidity rate of 15%. The authors advocated surgery within 24 hours of the subarachnoid hemorrhage, removal of blood clots from the basal cisterns, and enhancement of cerebrospinal fluid drainage by placement of ventriculocisternal drains.

In a series of 64 subarachnoid hemorrhage patients, Mizukami et al. (117) operated within 4 days of hemorrhage. The surgical approach was guided by CT findings in order to remove as much blood clot as possible. In 90% of cases, successful removal of clots from the basal frontal interhemispheric fissure, Sylvian cisterns, and the anterior insular cisterns was achieved. Angiography performed between days 6 and 15 post-hemorrhage revealed no or only mild

vasospasm in the patients from whom clot was successfully removed as shown by CT scan. Similarly, these patients suffered no neurological deterioration. Permanent neurological deficits due to vasospasm occurred in 8 patients from whom complete clot removal was not accomplished, as demonstrated by post-operative CT scans.

Another series of 239 patients studied by Taneda lent further support to a belief in the beneficial effect of complete clot removal (138). Delayed ischemic deficits occurred in 25% of patients in whom surgery was delayed more than 10 days and in 28% of patients who underwent aneurysm clipping but incomplete clot removal. Only 11% of the patients who were operated upon within 48 hours of the hemorrhage and who underwent aggressive removal of subarachnoid clot developed delayed ischemic deficits.

Taneda et al. (183) reported a single case in which the patient was operated upon within 24 hours of the subarachnoid hemorrhage. Extensive removal of clot was performed on the side of surgery. Delayed ischemia developed 8 days after the subarachnoid hemorrhage on the ipsilateral side to surgery. This resolved with hypervolemic therapy. On day 16 post-subarachnoid hemorrhage, following complete recovery, vasospasm occurred on the contralateral side to surgery where the clot had not been evacuated. Massive infarction ensued and the patient was left with a severe deficit.

Clinical studies of the efficacy of clot removal are difficult to compare. Differences in surgical expertise, bias in interpreting outcome, and inability or unwillingness to randomize clot removal and non-removal strategies have inhibited the conduct of a definitive, prospective, randomized trial.

In the event that thorough clot removal cannot be accomplished at the time of surgery, alternative methods of removing residual clot have been devised. Subarachnoid cisternal lavage, both as a means of primary clot removal and as an adjunct to surgical clot evacuation, have met with varying success (68, 78, 115, 123, 147, 148). A recent study by Alexander et al. (2) evaluated the effect of cisternal lavage 24 hours after subarachnoid hemorrhage in the 2-hemorrhage dog model. While lavage was effective in removing the majority of gross clot, it was not effective in preventing vasospasm. The authors suggested that any postulated interaction of clot and cerebral vessels resulting in vasospasm must occur within the first 24 hours following the hemorrhage. Some clot did remain in contact with the cerebral vessels following the simple lavage and this may have been sufficient to initiate the vasospasm at a time later than 24 hours. In an effort to facilitate mechanical washout of clotted subarachnoid blood, Yoshida et al. (217) added the plasminogen activator urokinase to the lavage solution. Patients underwent irrigation of the subarachnoid space for 5 to 7 days with 2,000 ml of a solution of urokinase, 48 IU/ml. On CT scan, high densities in the premesencephalic cistern disappeared within 7 days while they were still present in patients irrigated with physiological saline only. In one patient who was treated by pooling 10 ml of urokinase solution, 1,200 IU/ml, in the subarachnoid space for 10 minutes, followed by irrigation, high CT densities disappeared by 4 days.

Summary

The development of cerebral vasospasm in a patient who has survived the initial devastation of a subarachnoid hemorrhage is a frustrating and discouraging problem. Successful repair of a ruptured cerebral aneurysm and the lack of neurological deficit post-operatively are no guarantee that the patient will not have a poor result two weeks post-hemorrhage. Infarctions causing severe neurologic deficits or death are not rare. While much effort has been expended attempting to find the specific agent causing vasospasm, there have been no definitive results. The numerous therapeutic modalities that have been tried attests to the lack of success in finding an effective treatment.

Experimental and clinical trials showed promise in the use of the calcium blocker nimodipine in treating vasospasm and delayed ischemia. More recently, its effectiveness has been questioned. Primate studies at low dosage failed to give definitive results.

The role of aggressive clot removal has also been examined. Similarly, conclusive evidence of its effectiveness has been lacking.

The studies described in this thesis were designed in an attempt to shed some light on these two areas. The roles of both higher oral dose nimodipine and aggressive early clot removal were examined in a primate model of chronic cerebral vascular spasm after subarachnoid hemorrhage.

CHAPTER TWO: THE PRESENT STUDIES

I. Objectives and Experimental Design

The basis of this thesis rests in two experimental studies:

A: effects of nimodipine on chronic cerebral vasospasm, and,

B: effects of early clot removal on chronic cerebral vasospasm.

Each of these investigations includes 2 or more studies which evaluate separate areas of the problem.

All of the experiments reported here were evaluated and approved by The Animal Ethics Committee of the University of Alberta. All animal care and surgical procedures conformed to the standards set out by the Canadian Council on Animal Care.

STUDY A: Nimodipine and Chronic Vasospasm

i. Clinical, radiological, and pathological findings.

ii. Pharmacological *in vitro* studies of vessels in spasm.

iii. Pharmacological studies of *in vitro* effects on cerebral arteries of monkey, man, and dog.

iv. Nimodipine infusion and cerebral flood flow.

Objectives

1. Evaluate the efficacy of oral, high dose nimodipine [isopropyl - (2-methoxyethyl) - 1,4-dihydro-2,6-dimethyl-4 (3-nitrophenyl) - 3, 5-pyridinedicarboxylate] in the

prophylaxis of chronic cerebral vasospasm and delayed ischemic deficits.

2. Evaluate the effects of oral nimodipine and chronic vasospasm on hemispheric blood flow.
3. Examine the extent of structural and morphological changes in cerebral arteries in spasm at 7 days.
4. Examine the reactivity of cerebral arterial segments in the presence or absence of vasospasm and determine whether chronic administration of nimodipine can influence the contractility.
5. Compare the reactivity of cerebral arterial segments of monkey, man, and dog both with and without nimodipine to various standard agonists.
6. Examine the effect of intravenous nimodipine infusions on cerebral blood flow in the monkey.

Design

Study A(*i*) - This study was designed as a randomized, blinded, placebo-controlled trial. Restricted randomization was performed within 2 groups of 12 animals to minimize the effects of bias due to improvement of surgical technique over the course of the experiment. Three pilot animals underwent the surgical procedures to allow refinement of the technique prior to beginning the experiment.

Thirty female cynomolgus monkeys (*Macaca fascicularis*) were entered into the study and were subjected to subarachnoid hemorrhage induction. Six animals died within 24 hours post-operatively and were excluded from the study. The 24 survivors were randomized to one of 4 treatment groups of 6 animals each. Each treatment group

received one of nimodipine 3, 6, or 12 mg/kg q8h for 7 days, or placebo q8h for 7 days. The randomization code was not broken until all data had been analyzed. Two additional animals underwent the surgical procedure except for the actual placement of the blood clot. They thus acted as sham controls for the surgical procedures. Variables measured or observed at each stage included general physical and neurological status, mean arterial blood pressure, cerebral blood flow, size of induced subarachnoid hemorrhage and angiographic cerebral vessel caliber. After 7 days of treatment, the animals underwent repeat evaluation of parameters and were then randomized within groups to one of two subgroups: pathology or pharmacology. The pathology subgroup underwent *in vivo* fixation of the brain for pathologic examination of the cerebral vessels. The pharmacology subgroup was sacrificed by exsanguination for *in vitro* evaluation of the contractile responses of the cerebral vessels (Study A*ii*).

Study A(*ii*) - The pharmacology subgroup's middle cerebral arteries from study A*i* were prepared for *in vitro* evaluation of their contractile responses to potassium, norepinephrine, and 5-hydroxytryptamine. Preparation was performed in a single-blind manner in that the nimodipine treatment regime was unknown when the isolated tissues were examined. Three replicates were studied for each artery and three animals were examined from each nimodipine/placebo treatment group.

Study A(*iii*) - To further characterize the primate model, and to determine the direct *in vitro* effect of nimodipine on cerebral arterial contractions, middle cerebral and basilar arteries were

obtained from healthy normal monkeys, and mongrel dogs. Human arteries were also obtained at autopsy within 18 hours of death from causes other than subarachnoid hemorrhage.

Arteries were prepared for *in vitro* analysis as in study A(*ii*) with the addition of testing the agonists prostaglandin $F_{2\alpha}$ and hemoglobin. The samples were then randomized to one of two groups. One group had nimodipine in concentrations of 10^{-10} to 10^{-7}M added to the organ baths and were then allowed to equilibrate for one hour. Dose effect curves were then re-determined using the second group as time controls.

Study A(*iv*) - In order to determine the effect of intravenous nimodipine infusions on cerebral blood flow, 6 female monkeys were randomly allocated to one of 3 groups: nimodipine 2 μg/kg/min, 5 μg/kg/min, or placebo. Each animal was evaluated for its cerebral blood flow response to $PaCO_2$. Infusions of the appropriate dose of nimodipine were then started and cerebral blood flow was measured every 15 minutes until it remained unchanged for 2 consecutive determinations and until the animals had received the infusion for at least 1 hour. The animals were allowed to recover post-operatively for at least 1 week and were then returned to the operating room to be retested in an alternate group. This was repeated until all animals had been evaluated in each experimental group.

STUDY B: Clot Removal

i Clinical, radiological, and pathological findings.

ii. *In vitro* evaluation of cerebral arteries from B(*i*) for basal prostacyclin, thromboxane A_2 and leukotriene C_4 synthesis.

Objectives

1. Evaluate the role of clot removal 24 hours post-subarachnoid hemorrhage in the prophylaxis of cerebral vasospasm and delayed ischemic deficits.
2. Develop the primate model of subarachnoid hemorrhage to increase the incidence of delayed ischemic deficits.
3. Evaluate and compare the basal production of prostacyclin, thromboxane A_2 and leukotriene C_4 of monkey cerebral vessels in spasm and not in spasm.

Design

Study B(*i*) - The efficacy of complete clot removal 24 hours after subarachnoid hemorrhage in the prevention of chronic cerebral vasospasm was evaluated in monkeys in a blind, randomized, controlled trial. Twenty-four female cynomolgus monkeys (*Macaca fascicularis*) were randomized to one of three groups: sham, clot, or clot-removal. The major cerebral vessels were dissected free of arachnoid bilaterally. An average 4.97 g autologous hematoma was placed around the vessels in the subarachnoid spaces in the clot and clot-removal groups. Saline was instilled in the subarachnoid spaces of the sham group. All animals were re-operated upon 24 hours after the first procedure. The clot-removal group underwent complete evacuation of the hematoma. The sham and clot groups were simply re-closed after 3

hours of anaesthesia. Indices monitored before and 7 days after subarachnoid hemorrhage induction included neurologic status, angiographic cerebral vessel caliber and arterial blood pressure. All animals were evaluated with magnetic resonance imaging; representative animals were evaluated with CT brain scans.

Animals were sacrificed by exsanguination on day 7. The cerebral vessels were rapidly removed and were used in Study B(*ii*).

Study B(*ii*) - The cerebral vessels from Study B(*i*) were rapidly removed from the animals, cleaned of connective tissue and blood, cut into 1 mm sections, placed in weighed vials, and frozen at -70°C until processed. Measurement of the stable metabolites of prostacyclin (6-keto-prostaglandin $F_{1\alpha}$) and thromboxane A_2 (thromboxane B_2) was done by commercially available radioimmunoassay kits. Testing was carried out in a blinded manner and was performed on individual, unilateral main arterial trunks (left and right middle cerebral and posterior cerebral arteries, as well as anterior cerebral and basilar arteries).

Preliminary testing of monkey cerebral arteries revealed that leukotriene C_4 levels were below the limits of detection (< 25 pg) and therefore this portion of the study was dropped from the main testing sequence.

II - MATERIALS AND METHODS

Preparations were similar for both series of studies with respect to the surgical and pharmacological procedures, so the techniques will be described together. Differences in procedure between the series will be noted where appropriate.

Animal Preparation

Adult female cynomolgus monkeys (*Macaca fascicularis*)(Charles River Primate Research Corp., Port Washington, New York) with an average weight of 3.4 kg (range 2.8 to 4.2 kg) were used in the studies. Anaesthesia was induced with ketamine hydrochloride 6 to 10 mg/kg i.m. for baseline and sacrifice evaluations or with sodium pentobarbital 26 mg/kg i.v. for subarachnoid hemorrhage induction and clot removal. Animals were intubated with a 4.5 to 5.5 mm uncuffed endotracheal tube and were ventilated with a 2:1 mixture of N_2O:O_2 administered with a variable phase animal respirator (Harvard Apparatus, Inc., Millis, Massachusetts). Ventilation was adjusted by volume to maintain $PaCO_2$ near 39 mm Hg (30 to 35 mm Hg during craniotomy) or as indicated for cerebral blood flow studies. Intravenous infusions were administered through a percutaneous 22 gauge catheter in a saphenous or cephalic vein. Paralysis was induced and maintained with gallamine 2 mg/kg i.v. q45 minutes. Procaine penicillin, 100,000 IU/kg i.m. was given 30 to 60 minutes before any major procedure. Body temperature was maintained at 37°C by a heating pad placed beneath the animal which was monitored and

controlled by a rectal thermometer and thermostat (Tele-thermometer; Yellow Springs Instrument Corp., Yellow Springs, Ohio). The operative areas were shaved and prepared with Betadine surgical scrub and solution.

With sterile technique, the femoral artery on either side was exposed by cut down and was catheterized with a 5-French radiopaque polyethylene catheter (39) through a transverse arteriotomy between two ligatures. Xylocaine 2% was injected subcutaneously and into the femoral sheath to prevent femoral arterial constriction. The catheter was advanced under fluoroscopic control until the tip was in the innominate artery. Position was confirmed by the opacification of both common carotid arteries following a 0.5 to 1.0 ml injection of contrast medium (iothalamate meglumine 60%). This catheter was also used for periodically obtaining arterial blood samples for determination of blood-gas analysis, pH and hematocrit values. The patency of the catheter was maintained by period flushing with 1 to 2 ml of 0.9% saline with heparin, 10 IU/ml, to prevent clotting. The catheter was connected by way of a 3-way stopcock to a pressure transducer for monitoring arterial blood pressure (Statham P23dB pressure transducer; Statham Instrument Co., Oxnard, California), a Cordis injector for administering iothalamate meglumine, 60% (Cordis Corp., Miami, Florida), and an automatic injector to administer Xenon-133, for cerebral blood flow determination (Xenon injector, built at the University of Alberta). A Beckman Dynograph R611 eight channel recorder was used to record arterial blood pressure. This was calibrated against a mercury manometer immediately prior to each

experiment and was checked periodically throughout each experiment for drift.

At the termination of each chronic experiment, the catheters were removed and the ligatures tightened. No attempt was made to maintain patency of the femoral artery. All surgical incisions were irrigated with topical Bacitracin solution. Paralysis was reversed with prostigmine 0.07 mg/kg i.v. and atropine 0.02 mg/kg i.v. was given. The animals were ventilated with 100% O_2 until breathing spontaneously. They were extubated upon return of the gag reflex.

Subarachnoid Hemorrhage Induction

Study A: The animal's head was placed in a 3-point fixation vise with the right side up. A right frontotemporal skin flap, centered at the pterion, was created with a cutting cautery. A sigmoid incision was made, beginning at the anterior zygoma, curving posteriorly and superiorly, then terminating anteriorly parallel to the sagittal suture about midway along the supraorbital ridge. A U-shaped muscular flap was then turned posteriorly using cutting cautery. A 1 cm trephine and a Cloward rongeur were used to create a 1.5 cm craniectomy. Care was taken to ensure the inferior portion of the craniectomy was on the floor of the middle fossa and that the sphenoid ridge was adequately removed. Bone wax was applied to stop the bleeding from the diploic veins. The middle meningeal vessels were cauterized and cut as the dura was opened in a semicircular fashion with the concavity toward the sphenoid ridge. The dural flap was sutured open to the subcutaneous tissues. The temporal lobe was

gently retracted posteriorly with a self-retaining retractor until the origin of the Sylvian fissure was visualized. Using sharp and blunt dissection, the arachnoid membrane over the intradural internal carotid artery, posterior communicating artery, anterior cerebral artery and the sphenoidal segment of the middle cerebral artery was opened widely. An autologous blood clot averaging 6.4 ml was then carefully placed around the exposed arteries in the subarachnoid space. The dura was then closed in a watertight fashion with 6-0 silk. The muscular flap was replaced and the galea was closed with 2-0 silk and the skin was sutured with 3-0 monofilament polyethylene (Dermalon; Davis & Geck, New York, New York) using vertical, interlocking, mattress sutures .

Sham animals underwent identical procedures except that in place of an autologous blood clot, 6 ml of normal saline were instilled into the subarachnoid space.

Study B - The basic subarachnoid hemorrhage induction in Study B was similar to that in Study A. Following the initial opening of the arachnoid, retraction was shifted posteriorly to raise the temporal lobe superiorly. The 1 mm Sugita tapered retractor was used to expose the posterior communicating artery along its length. Liliequist's membrane was incised and the arachnoid over the tip of the basilar artery and along the posterior cerebral artery was opened. The autologous blood clot was placed to include these vessels as well, and the incisions were closed as described (Fig. 1).

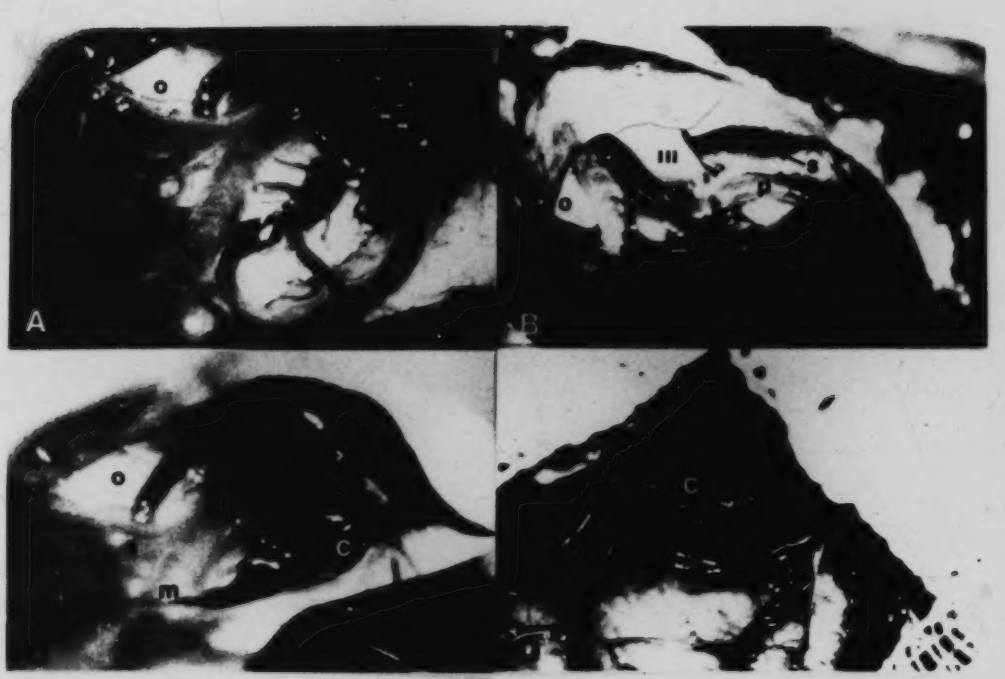


Fig. 1. Photomicrographs of the subarachnoid hemorrhage induction procedure. A, initial dissection of the internal carotid, anterior cerebral and middle cerebral arteries. B, the dissection is continued posteriorly to the posterior cerebral and superior cerebellar arteries. C, initial placement of small, autologous blood clot fragments. D, blood clot *in situ*. a, anterior cerebral artery; c, blood clot; i, internal carotid artery; m, middle cerebral artery; o, optic chiasm; p, posterior cerebral artery; s, superior cerebellar artery, III, oculomotor nerve.

The animal was then repositioned and the procedure was repeated on the left side. Total bilateral clot placed averaged 4.97 grams in weight.

Clot Removal

Study B only - Twenty-four hours after the subarachnoid hemorrhage induction, the animals were returned to the operating room. The incisions were reopened. The animals in the clot-removal group underwent meticulous removal of the previously placed hematomas. All fragments of removed hematoma were saved for weighing. The subarachnoid spaces were flushed with copious amounts of normal saline until all traces of blood were removed. The incisions were then closed as previously described.

The sham and clot groups were merely opened and closed with no manipulation of the hematoma or cerebral vessels. They were maintained under anaesthesia for an average of 3 hours to duplicate the experience of the clot-removal group.

Cerebral Angiography

The radiographic equipment used for the cerebral angiography has been described elsewhere (140).

Retrograde cerebral angiography was performed through the 5-French sigmoid tip catheter which was inserted in the femoral artery. One arterial phase anteroposterior film was obtained as a baseline prior to subarachnoid hemorrhage induction. A second film was obtained 7 days after the hemorrhage was induced.

The X-ray beam was directed parallel to Reid's baseline and was centered at the nasion. Exposures of 70 KeV at 2.5 mAs and 1/180 second were obtained. The angiograms were obtained by injecting 10 ml of iothalamate meglumine, 60%, at 300 psi which required approximately 1 second time. Subtraction films were processed for each angiogram taken. Magnification factors were kept constant by maintaining a table to film distance of 78 cm and a subject to film distance of 18 cm. As well, a radiopaque magnification control device was used to correct for any variation in film, exposure, or development (140).

Vessels were measured bilaterally at 4 points: the extradural internal carotid artery proximal to the cavernous sinus; the intradural internal carotid artery between the posterior communicating and ophthalmic arteries; the anterior cerebral artery; and the proximal sphenoidal middle cerebral artery. The proximal and distal pericallosal artery was also measured. Caliber at each of the arterial sites was measured 6 times with a calibrated optical micrometer. A mean value was calculated for each site. Serial measurements were recorded as percentage change from baseline values in individual animals and by group. Degree of spasm was graded as follows: 0% to -10% = no vasospasm; -11% to -30% = mild vasospasm; -31% to -50% = moderate vasospasm; and > 50% reduction = severe vasospasm.

The angiographic appearance of collateral circulation on Day 7 was also graded as follows: 0 = no collateral circulation seen; + = appearance of small new channel or dilation of a collateral channel detected on baseline angiography; ++ = one or more

medium-sized new channels; and +++ = one or more large-sized new channels.

Cerebral Blood Flow

Study A(*i*) - Cerebral blood flow was measured twice before cerebral angiography at baseline and on Day 7 post-hemorrhage. Bilateral cerebral blood flow was determined by injecting 1.0 mCi Xenon-133 intra-arterially through the femoral catheter. Radioactivity was recorded from one 2.5 cm diameter NaI-Tl collimated scintillation detector placed over each dorsolateral-frontoparietal brain region. The $PaCO_2$ was maintained at 40 ± 1 mm Hg. The cerebral blood flow was calculated by the initial slope index method and was corrected to a $PaCO_2$ of 40 mm Hg.

Study A(*iv*) - Xenon-133 was administered as above, however, isotope clearance was measured by four 1 cm diameter NaI-Tl collimated detectors arranged around the coronal suture on the skull. As well, bilateral neck dissections were performed and temporary clips were placed at the origins of the external carotid arteries.

The methods of dispensing, administering and detecting the Xenon-133 as well as for calculating cerebral blood flow as performed in this laboratory have been reported in detail elsewhere (17, 135, 142).

Drug Administration

The nimodipine and placebo were prepared locally (Dr. J. A. Rogers). Nimodipine is sensitive to white light, especially in solution (27), therefore all preparations were performed and

dispensed under gold light (F-15T8-60-gold, Westinghouse Electric, Pittsburgh, Pennsylvania). The drugs were stored in amber bottles, at 5°C, until immediately prior to use.

The animals were lightly sedated with ketamine 4 to 6 mg/kg and the drug was administered via a nasogastric tube. The drug administration room was equipped with gold light. This served to protect the administered drug from white light and prevented identification of the given drugs. The randomization code was not broken until all the data had been analyzed.

Serum and cerebrospinal fluid samples were drawn from all animals receiving nimodipine or placebo just prior to sacrifice. Nimodipine levels were determined by high performance liquid chromatography.

Neurological Assessment

Each animal underwent neurological assessment prior to and within 8 hours of subarachnoid hemorrhage induction. They were then examined at least daily until sacrifice on Day 7. A five division neurological grading system was used for evaluation.

Grade 1: active, vocal, normal

Grade 2: lethargic, upright, but unsteady, less active or vocal, but no significant neurological deficit (paresis, paralysis)

Grade 3: no spontaneous attempt to stand upright but responding to stimulation, moderate neurological deficit (monoparesis, hemiparesis)

Grade 4: severely obtunded, no response to stimulation, severe neurological deficit (monoplegia, hemiplegia, quadriplegia)

Grade 5: moribund, failing vital signs, no response to stimulation.

Computed Tomography and Magnetic Resonance Imaging

Computed tomographic (CT) imaging was performed either on a Picker SS03 or General Electric 9800 CT scanner. Magnetic resonance imaging (MRI) scans were performed on a Bruker BNT 100 NMR imaging system obtaining proton images at 100 MHz. A spin-echo technique was employed using a repetition time TR=3s. Eight echoes were obtained at intervals of TE=32msec for each slice imaged, allowing careful comparison of affected and unaffected regions of the brain at the same location at the various stages of the study.

Animals were sedated for all scans with sodium pentobarbital, i.v., titrated individually to achieve light anaesthesia, and supplemented as required. Animals were intubated in order to assure airway patency during the period of neck flexion required for MRI scanning. All animals received MRI scanning on Day 7 prior to sacrifice. Animals were chosen at random from each group for baseline, post-subarachnoid hemorrhage and post-clot removal MRI and CT scans. Any animal demonstrating a clinical neurological deficit was scanned, with the exception of monkey #125 which died before scanning could be performed.

Animals in Study A(*i*) received CT scans if and only if they developed a neurological deficit.

Pathology

Study A(*i*) - Animals randomized to the pathology subgroup underwent intra-arterial cerebral fixation following the Day 7 angiogram. Under general anaesthesia and mechanical ventilation, a left thoracotomy was performed, cutting through the fifth intercostal space. Heparin (3,000 I.U.) was injected i.v. to facilitate washout of the blood. The ascending aorta was cannulated through a purse string suture in the left cardiac ventricle, the right atrium was widely opened, and the descending aorta was ligated. Normal saline was infused through the aortic cannula at 110 mm Hg pressure and 23°C until the atrial return was clear (this required about 200 ml of saline). The perfusion solution was immediately changed to a 2% glutaraldehyde, 2% formaldehyde in Millonig's buffer, 0.12 M, pH 7.4, at 4°C. Approximately 1 litre of this solution was infused over 5 to 10 minutes at 110 mm Hg.

The brain was immediately removed and placed in the fixation solution for a minimum of 24 hours. With the aid of an operating microscope, the cerebral arteries (basilar and bilateral middle cerebrals) were carefully resected and each was divided into 3 segments. Each segment was placed in a separate labelled vial of fixative and was processed for scanning electron, transmission electron, or light microscopy. The brains were sectioned and prepared for light microscopy.

Study B(*i*) - All animals were exsanguinated under general anaesthesia. The brains were immediately removed, the vessels resected for pharmacological study, and the brains placed in 10%

buffered formalin. The brains were allowed to fix for 2 weeks after which they were sectioned and prepared for light microscopy.

Light Microscopy

After fixation, the tissues were dehydrated through a graded ethyl alcohol series and cleared in xylene. Tissues were transferred to an incubator (58°C) and were moved through 2 changes of paraffin (Tissue Prep embedding pellets; Fisher Scientific) at 56.5°C. The blocks were sectioned at 8 μm on a steel knife in a rotary microtome, floated onto albuminized slides, and allowed to dry overnight at 40°C. The slides were stained with Harris' hematoxylin and counterstained with alcoholic eosin Y. Photography was done through a Zeiss microscope (Standard 14 laboratory light microscope, model D-7082; Zeiss, Oberkochen, West Germany).

Electron Microscopy

After fixation, the tissues were washed for 45 minutes through 3 changes of Millonig's buffer, 0.13 M and were re-fixed in 1% osmium tetroxide in Millonig's buffer 0.07 M. They were then washed for 30 minutes through 3 changes of distilled water and were dehydrated through a graded series of ethyl alcohol.

Samples for scanning electron microscopy were transferred in absolute ethanol to a CO_2 critical point dryer (Seevac Inc., Pittsburgh, Pennsylvania) to be dried. They were mounted on aluminum stubs and sputter coated with gold (model S150B; Edwards; Crawley, West Sussex, England). They were examined in a scanning electron

microscope at 25 kV (Phillips model 505, Gloeclampenfabrieken, Eindhoven, The Netherlands).

Samples for transmission electron microscopy were transferred in absolute alcohol through 3 changes of propylene oxide for 30 minutes. They were fixed in propylene oxide: aryldite (CY212) epoxy resin; 1:1, for 4 hours and were then embedded in resin blocks. These were allowed to cure at room temperature for 24 hours and were then polymerized for 48 hours at 60°C. Sections were cut on an ultramicrotome (Reichert-Jung Ultracut) and were mounted on 300 mesh copper grids. Specimens were counterstained with uranyl acetate and lead citrate. They were examined in a transmission electron microscope at 80kV (Phillips model 410).

Pharmacology

Study A(ii) - After the animals of the pharmacology subgroup were sacrificed by exsanguination under general anaesthesia, the middle cerebral and basilar arteries were immediately resected and placed in oxygenated Krebs' bicarbonate solution of the following composition: Na^+, 132 mM; K^+, 5.9 mM; Ca^{++}, 2.5 mM; Mg^{++}, 1.2 mM; Cl^-, 122.7 mM; HCO_3^-, 25 mM; SO_4^{--}, 1.2 mM; $H_2PO_4^-$, 1.2 mM and dextrose, 11 mM. Each artery was cut into ring sections 3 mm in length which were suspended in organ baths of 10 ml working volume on stainless steel wires. The Krebs' solution was maintained at 37°C and was gassed with 95% O_2 and 5% CO_2. Contractions were recorded isometrically using Grass FT03 strain gauges connected to a Grass 7D polygraph.

The arterial rings were placed under 1 g tension and were equilibrated for 2 hours during which time the bathing solution was changed approximately every 15 minutes. Dose-effect curves were determined for the agonists 5-hydroxytryptamine, norepinephrine, and potassium chloride.

These studies were conducted in a blinded fashion such that the nimodipine dosage regime was unknown when the isolated tissues were examined.

The two middle cerebral arteries were distinguished by a letter only, and the data were combined only when the study had been completed. Three replicates were studied for each artery, and three animals were used from each nimodipine treatment group.

Study A(*iii*) - Similar techniques were used as described above, with the addition of dog and human arteries. Dogs of either sex weighing 16 to 20 kg were first anaesthetized with a phenobarbital (32 mg/kg), and the basilar and middle cerebral arteries were removed and prepared as described. Human arteries were obtained at autopsy within eighteen hours of death from causes other than subarachnoid hemorrhage. The viability of the postmortem human tissues was demonstrated by the development of contractions of at least 2 g tension to all agonists tested.

Contractions were recorded to various doses of norepinephrine, 5-hydroxytryptamine, potassium chloride, prostaglandin $F_{2\alpha}$ and hemoglobin.

Samples were then randomly divided into 2 groups. Nimodipine in concentrations of 10^{-10}M to 10^{-7}M was added to the organ baths of the first group and allowed to equilibrate for 1 hour. Dose effect

curves were then repeated for each of the agonists tested with both sample groups. The second group acted as time controls for the nimodipine treated samples. Nimodipine solutions and baths containing nimodipine were protected from light to prevent any inactivation of the compound. Contractions were recorded in grams tension developed and were converted to percentages of maximal baseline tension developed.

Drugs used in this study were all obtained from the Sigma Chemical Company, except nimodipine which was kindly supplied by Miles Pharmaceuticals, West Haven, Connecticut, U.S.A.

Study B(*ii*) - After sacrifice by exsanguination under general anaesthetic of the animals in the clot-removal study B(*i*), the brains were immediately removed and the cerebral arteries were resected under an operating microscope. The vessels were cleared of connective tissues and blood. Each main branch of the circle of Willis was cut into 1 to 2 mm segments which were placed in a pre-weighed microcentrifuge tube, re-weighed to determine net wet vessel weight, and were stored at -70°C until use. Storage times varied from 72 hours to 6 weeks.

Frozen arterial segments were allowed to thaw to room temperature and 1 ml of Ca^{++}/Mg^{++} free phosphate buffered saline (pH 7.4) was added to each tube. The tubes were then incubated for 5 minutes at 37°C in a shaking water bath. The tubes were placed in an ultracentrifuge and spun for 1 minute at 8,000 g. The supernatants were discarded. One ml of pre-warmed Hank's balanced, salt solution with 20 mM HEPES (N-2-hydroxyethylpiperazine -N'-2-ethanesulfonic acid, pH 7.4, Gibco, Burlington, Ontario) was added to each tube and

the arterial segments were incubated for 30 minutes at 37°C with agitation. Following incubation, the tubes and segments were centrifuged for 1 minute at 8,000 g and aliquots of the supernatant were removed and frozen at -20°C for subsequent radioimmunoassay.

Basal levels of prostacyclin and thromboxane A_2 were measured in the arterial segments as their stable hydrolysis products, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 respectively, using commercially available radioimmunoassay kits (New England Nuclear, Lachine, Quebec). Separate standardization control curves were constructed for each series of specimens analyzed.

Activated charcoal was used to separate free and antibody bound fractions. The limits of detection for 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 were 10 pg and 5 pg respectively.

Data Analysis

Physiologic and Radiographic Data - Data for all variables were coded, entered into a computer and edited. Descriptive statistics and frequencies were computed for each variable at each time period. Angiographic vessel caliber and hemodynamic variables were compared with student's *t*-tests and relationships between variables were determined with Pearson's correlation coefficients. Intergroup comparisons and comparisons between Day 0 and Day 7 were by an analysis of variance or by an analysis of covariance if adjustments for different baseline values were needed.

The level of significance for all tests of comparison was $p < 0.05$ unless otherwise stated.

Pharmacologic Data - Contractions were recorded in grams tension developed. Data were coded, entered into a computer and edited. Comparisons between groups and within groups over time were done by an analysis of variance facilitated by a specifically designed computer program for handling dose-response analysis (28).

Data for the radioimmunoassay study was compared between groups by an analysis of variance.

The level of significance for all tests of comparison was $p < 0.05$ unless otherwise stated.

CHAPTER THREE: RESULTS

Study A - Nimodipine and Chronic Vasospasm

Study A(*i*) - Clinical, Radiological and Pathological Findings

Twenty-three of the 24 animals assigned to treatment groups survived to the end of the study. One animal in the placebo group died on Day 4 from septicemia after an isolated wound infection.

Comparisons within each group and across the four groups showed no significant differences in baseline values for body weight, measured physiological indices (pH, $PaCO_2$, PaO_2, hematocrit, mean arterial blood pressure, heart rate), cerebral blood flow, or cerebral vessel caliber. The groups were therefore combined for baseline statistical analysis (Table I).

Comparisons between groups showed no significant differences in Day 7 values for body weight, measured physiological indices, or cerebral blood flow. Comparisons across groups between baseline and Day 7 showed a significant decrease in mean arterial blood pressure with the nimodipine dose of 12 mg/kg ($p < 0.01$).

Comparisons within each group between baseline values and Day 7 values showed no significant differences for body weight, measured physiological indices (except hematocrit and mean arterial blood pressure), or cerebral blood flow.

There was a decrease in hematocrit in each group from Day 0 to Day 7 (mean $\pm$ SE, 37 $\pm$ 1 to 27 $\pm$ 1) with $p < 0.01$. However, there were no significant differences between groups.

TABLE I

Nimodipine and Chronic Cerebral Vasospasm

Values for Measured and Observed Indices ($\bar{x}\pm se$)[†]
For Each Treatment Group

Parameter		Pre-SAH	Day 7 Post-SAH			
			Placebo	3 mg/kg	6 mg/kg	12 mg/kg
No. of Monkeys		24	5	6	6	6
Body Weight		3.3±0.4	3.1±0.4	3.2±0.4	3.1±0.4	3.0±0.4
MABP (mm Hg)		102±18	92±22	94±13	83±13	80±26
HR (per min)		203±23	199±22	196±31	203±12	175±18
$PaCO_2$ (mm Hg)		38±1	38±1	38±1	38±1	40±1
Vessel Caliber						
ICA	1	1.4±0.1	1.1±0.1	1.3±0.1	1.3±0.1	1.3±0.1
	2	1.4±0.1	1.2±0.1	1.3±0.1	1.3±0.1	1.4±0.1
	3	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
	4	0.9±0.1	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.1
ACA	5	0.7±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1
	6	0.8±0.1	0.7±0.1	0.8±0.1	0.8±0.1	0.8±0.1
MCA	7	0.7±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1
	8	0.8±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.9±0.1
PCA	9	0.8±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1
	10	0.8±0.1	0.9±0.1	1.0±0.1	1.0±0.1	0.9±0.1
Hemispheric CBF (ml/100 g/min)						
Right		48±2	42±2	49±4	45±3	40±2
Left		47±2	50±2	53±4	54±3	45±3
Size of Hematoma (ml)(Day 0)		n/a[‡]	6.0±1.0	6.4±0.7	6.5±0.5	6.7±0.5

[†]MABP, mean arterial blood pressure; HR, heart rate; 1, right extradural internal carotid artery; 2, left extradural internal carotid artery; 3, right supraclinoid internal carotid artery; 4, left supraclinoid internal carotid artery; 5, right anterior cerebral artery; 6, left anterior cerebral artery; 7, right sphenoidal middle cerebral artery; 8, left sphenoidal middle cerebral artery; 9, proximal pericallosal artery; 10, distal pericallosal artery; CBF, cerebral blood flow.

[‡]not applicable

There was no significant difference between groups in the size of the hematoma.

All animals developed persistent moderate to severe diarrhea 1 to 2 days after treatment was begun.

Cerebral Vasospasm

Vasospasm was defined as a 10% or greater reduction in vessel caliber from the baseline value (39, 140, 142). Vasospasm was present in 23 of the 24 subarachnoid hemorrhage animals at Day 7. The 1 animal in which vasospasm did not develop was from the nimodipine, 6 mg/kg group.

Moderate vasospasm (31 to 50% reduction in vessel caliber) was present in three of five (60%) of the placebo group; four of six (67%) of the nimodipine, 3 mg/kg group; three of five (60%) of the nimodipine, 6 mg/kg group; and three of six (50%) of the nimodipine, 12 mg/kg group. Severe vasospasm (>50% reduction in vessel caliber) was present in one of six (17%) of the 12 mg/kg group. No vasospasm was present in the 2 sham-operated animals (Table II, Figs. 2-6).

The difference in vessel caliber between clot side and non-clot side was statistically significant within each group ($p < 0.001$). The difference in vessel caliber between clot side and non-clot side was not significant between groups ($p > 0.05$) (Fig. 7).

Comparisons between groups of the mean percentage change from baseline of the clot versus non-clot vessels (defined as the grouped average vessel caliber of the intradural internal carotid, middle cerebral, and anterior cerebral artery of the respective sides) showed no significant differences (Fig. 8).

TABLE II

DEGREE OF VASOSPASM DEVELOPED BY

TREATMENT GROUP: STUDY A(*i*)

Nimodipine Treatment Group	Number of animals developing vasospasm		
	Mild	Moderate	Severe[†]
Sham (n=2)	0	0	0
Placebo (n=5)	2	3	0
3 mg/kg p.o. q8h (n=6)	2	4	0
6 mg/kg p.o. q8h (n=6)	2	3	0
12 mg/kg p.o. q8h (n=6)	2	3	1

[†]mild, 11% to 30% reduction in vessel caliber: moderate, 31% to 50% reduction in vessel caliber; severe, > 50% reduction in vessel caliber.

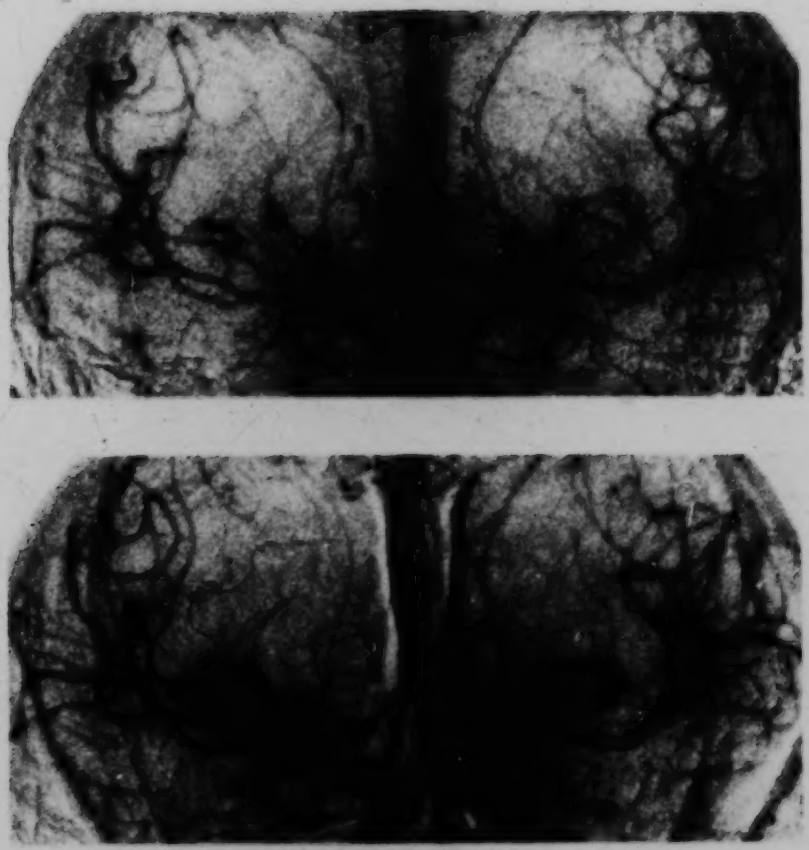


Fig. 2. Cerebral angiograms of a sham-operated animal (right side dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage.

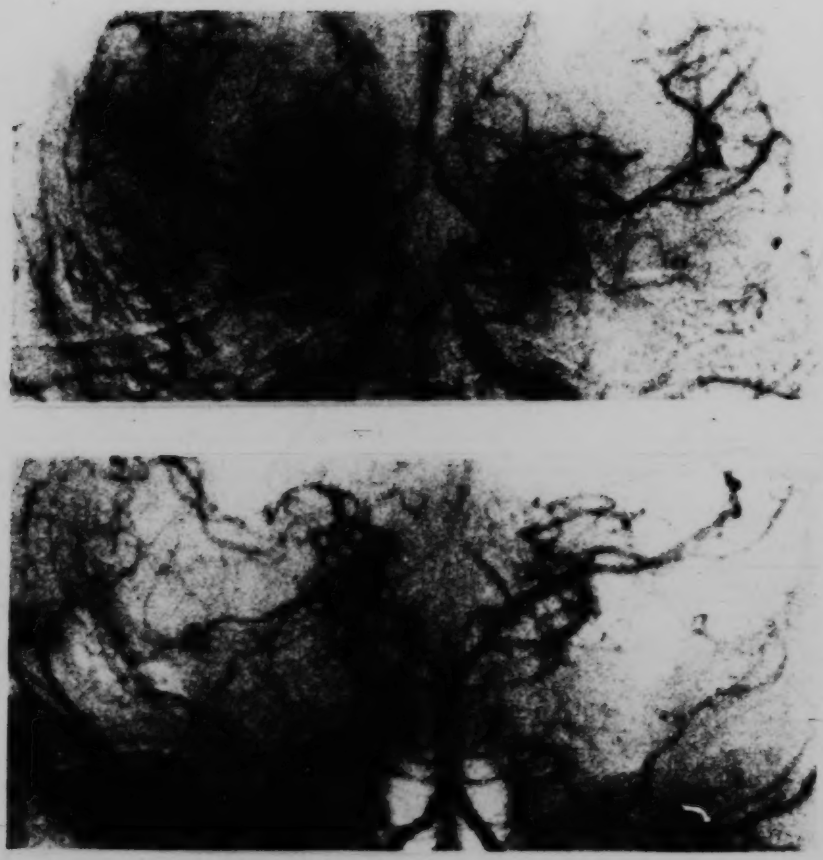


Fig. 3. Cerebral angiograms of a placebo-group animal (right side dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage.

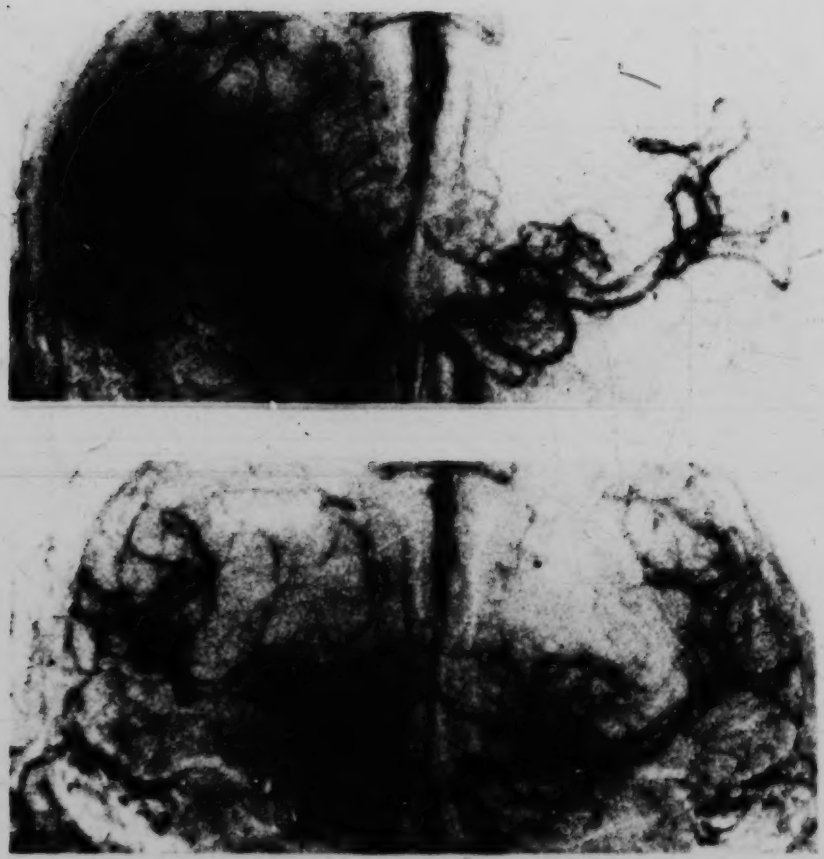


Fig. 4. Cerebral angiograms of a nimodipine, 3 mg/kg p.o. q8h group animal (right side dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage.

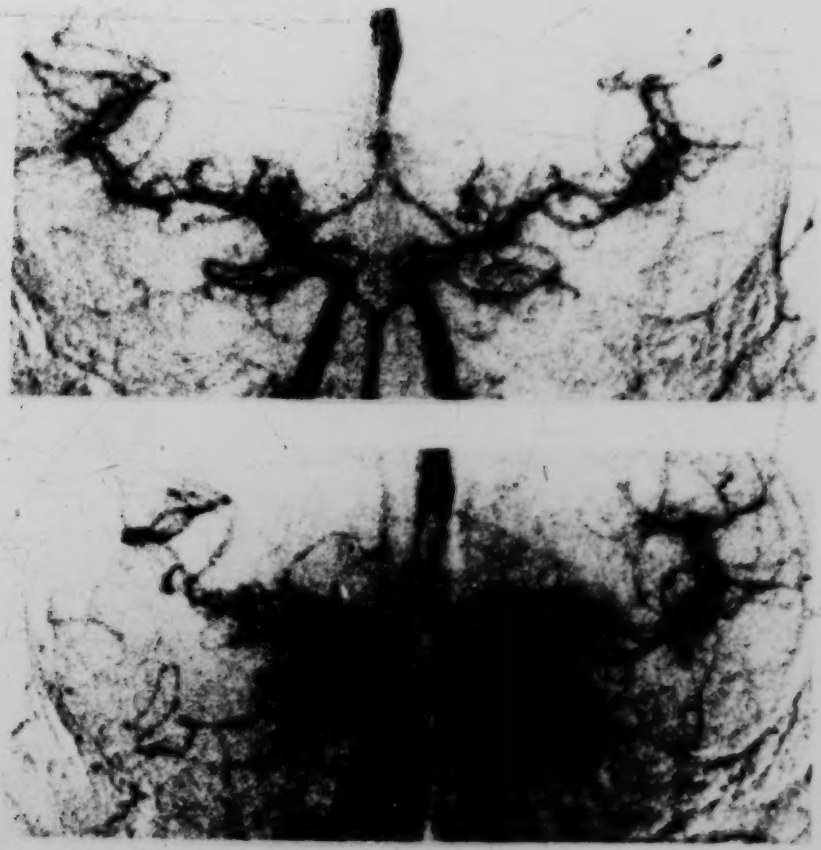


Fig. 5. Cerebral angiograms of a nimodipine, 6 mg/kg p.o. q8h group animal (right side dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage.

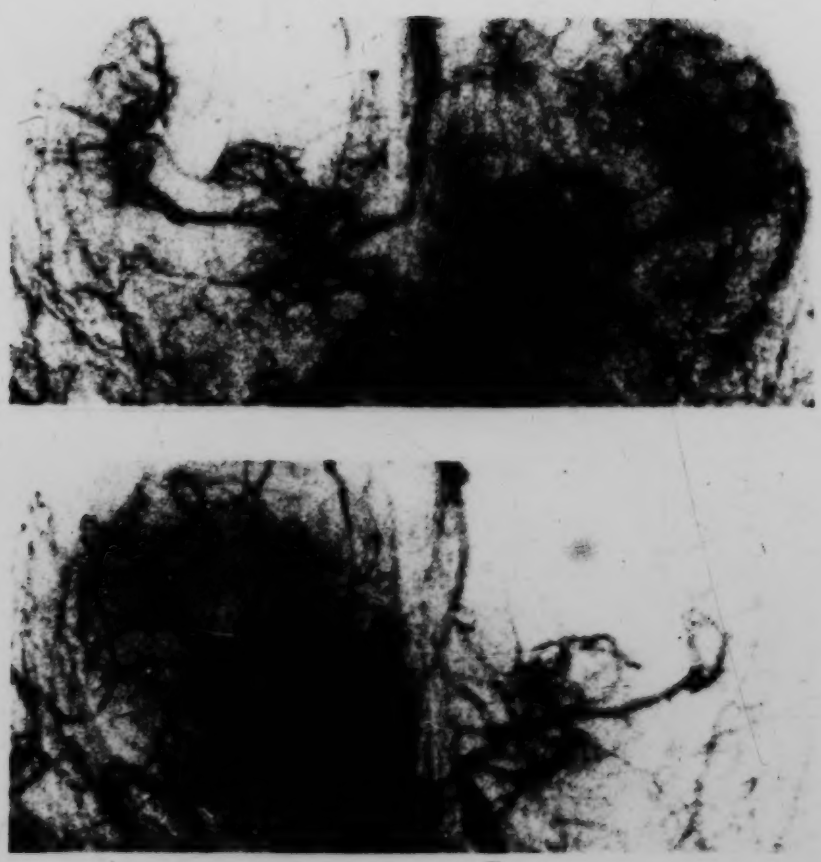


Fig. 6. Cerebral angiograms of a nimodipine, 12 mg/kg p.o. q8h group animal (right side dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage.

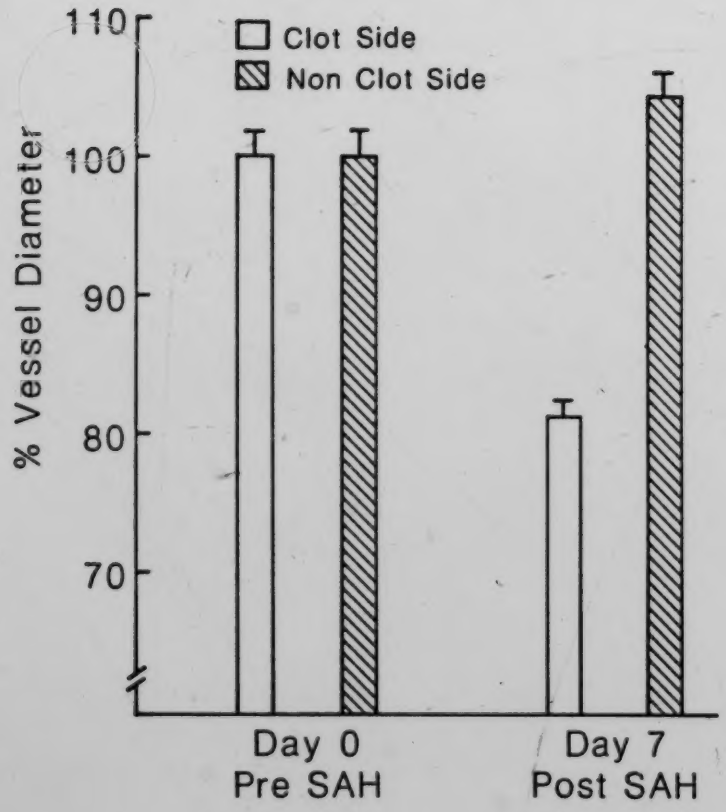


Fig. 7. Grouped average constriction of major cerebral vessels (intradural internal carotid artery, middle cerebral artery, and anterior cerebral artery) by side, pre-subarachnoid hemorrhage and on Day 7 post-subarachnoid hemorrhage.

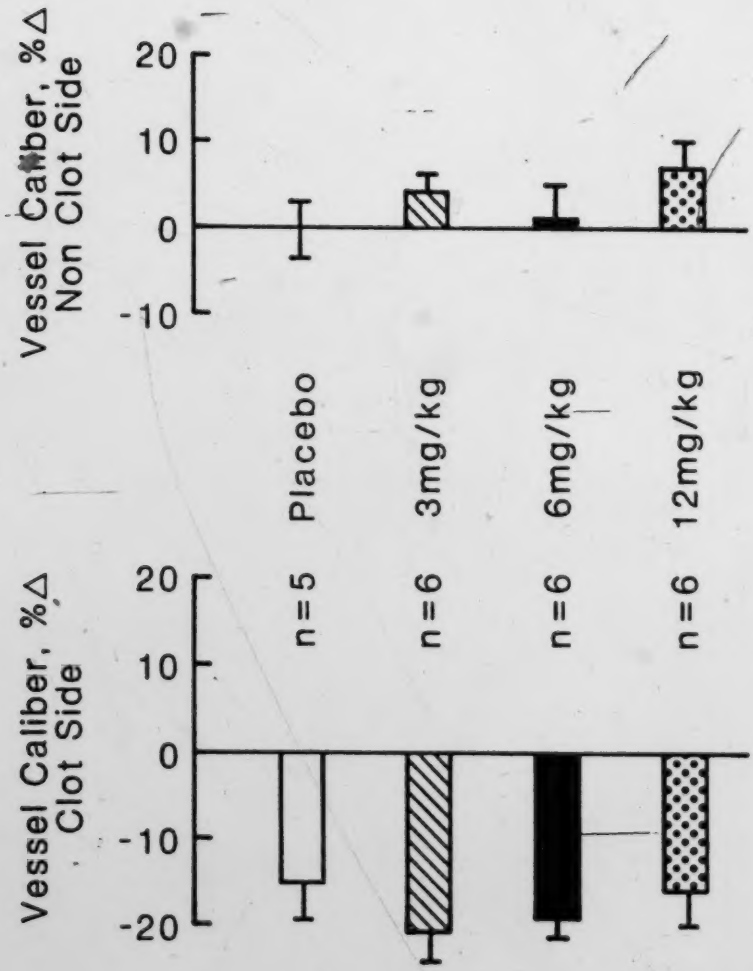


Fig. 8. Percentage change in vessel caliber from baseline value of major cerebral vessels (intradural internal carotid artery, middle cerebral artery, and anterior cerebral artery) by side and treatment group ($\bar{x}\pm se$).

Comparisons between groups for the presence of developed collateral circulation showed no significant difference (Table III).

Cerebral Blood Flow

No statistical differences in pre-subarachnoid hemorrhage or Day 7 post-subarachnoid hemorrhage cerebral blood flow values were detected within or between the four treatment groups.

Neurological Assessment

One animal receiving nimodipine, 12 mg/kg p.o., q8h, developed a delayed left-sided hemiparesis (contralateral to the side of clot placement) on Day 5. Over the course of 36 hours, the monkey developed progressive weakness of the left leg and arm, which was still present at sacrifice. Angiography on Day 7 revealed severe spasm of the right middle cerebral and internal carotid arteries (Figs. 9, 10). They were, however, patent. Brain sections clearly demonstrated an infarct in the territory of the right middle cerebral artery (Fig. 11).

Another animal, also receiving nimodipine, 12 mg/kg p.o. q8h, developed a weakness of the left leg beginning on Day 4, progressing until Day 6, and resolving by Day 7. Angiography on Day 7 revealed mild and moderate spasm of the right middle cerebral and internal carotid arteries, respectively. Brain sections did not demonstrate an infarct.

The remaining 22 treatment and 2 sham-operated animals demonstrated no focal neurological deficits.

TABLE III

DEVELOPMENT OF COLLATERAL CIRCULATION

BY TREATMENT GROUP

Nimodipine Treatment Group	Number of animals demonstrating collateral circulation†			
	0	+	++	+++
Sham	2	0	0	0
Placebo	1	2	2	0
3 mg/kg p.o. q8h	1	2	2	1
6 mg/kg p.o. q8h	2	2	1	1
12 mg/kg p.o. q8h	1	0	2	3

†0, no collateral circulation detected; +, appearance of small new channel or dilation of a collateral channel detected on baseline angiography; ++, one or more medium-sized new channels; +++, one or more large-sized new channels.

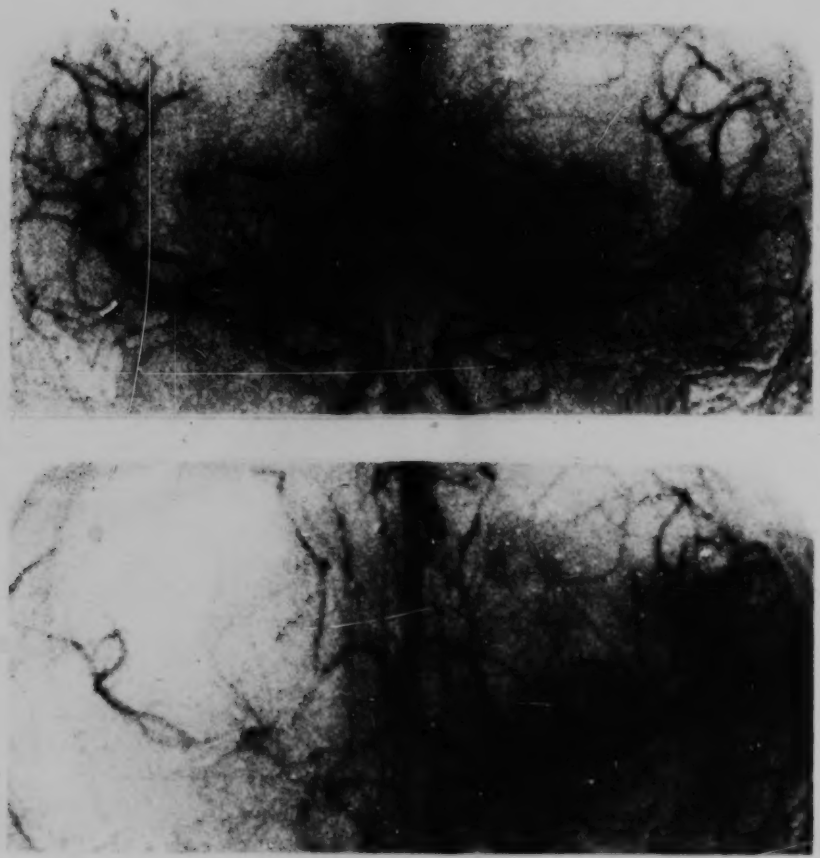


Fig. 9. Cerebral angiograms of Monkey No. 36 which developed a delayed neurological deficit on Day 5. *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage. Note the severe vasospasm of the right (clot side) cerebral vessels which extends down the right internal carotid artery.

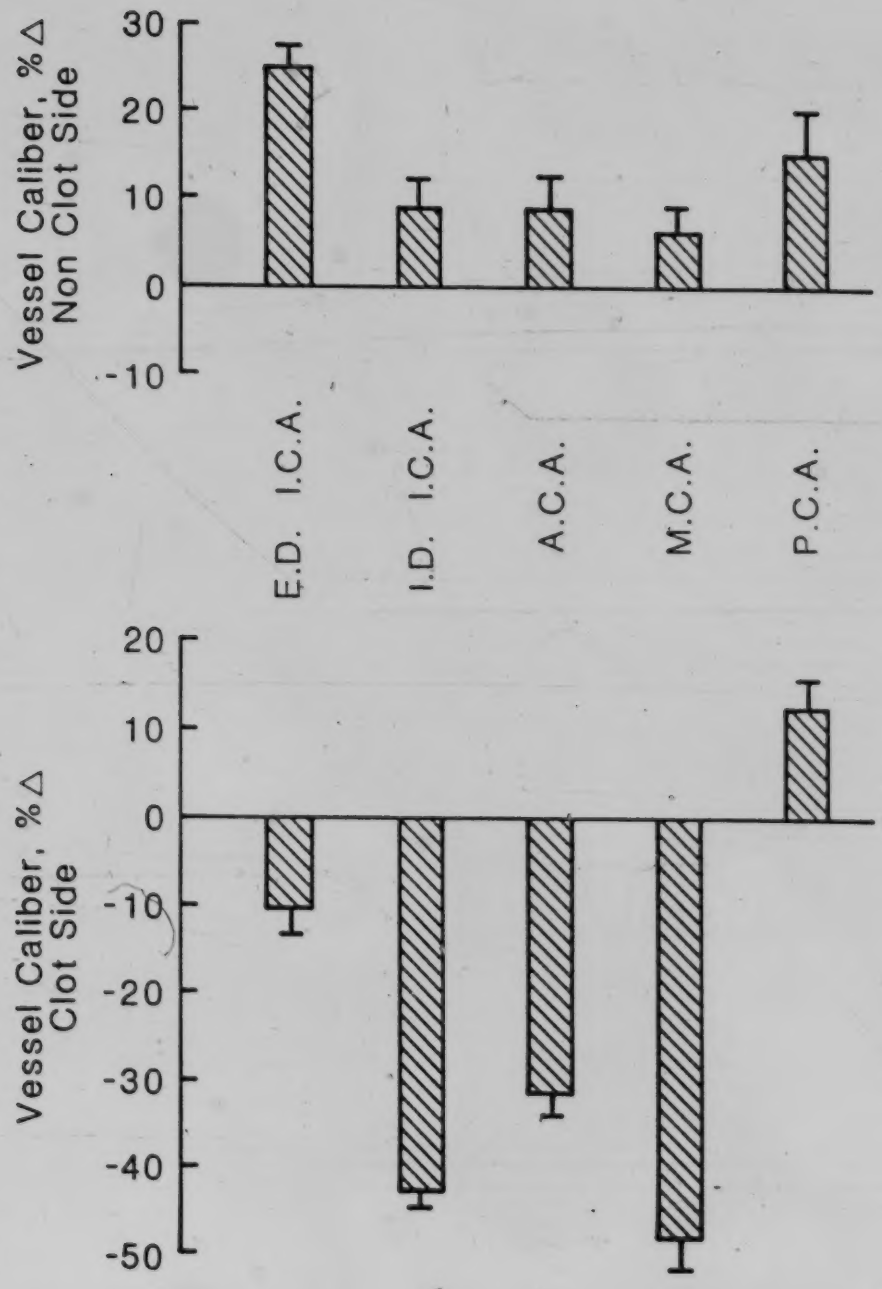


Fig. 10. Percentage change in angiographic cerebral vessel caliber from baseline of Monkey No. 36 (nimodipine, 12 mg/kg p.o. q8h). Bars represent standard errors (6 measurements).

Fig. 11. Axial brain section (hematoxylin and eosin stain) demonstrating infarction within the territory of the right middle cerebral artery of Monkey No. 36 (nimodipine, 12 mg/kg p.o. q8h).

Nimodipine Levels

Serum and cerebrospinal fluid nimodipine levels at time of sacrifice are shown in Table IV. There were no statistically significant differences in levels between groups.

Pathology

Pathological changes were, with few exceptions, not seen in control side arteries. Pathological findings present in the middle cerebral arteries on the clot side in 23 of the 24 animals included longitudinal endothelial convolutions, endothelial protrusion and areas of detachment, disruption of tight junctions, subendothelial swelling, vacuolization of endothelial and muscle cells, and muscle cell necrosis. There was no evidence of an adventitial inflammatory reaction. There was no difference in the incidence of pathological findings among the four treatment groups (Figs. 12-14).

The animal that did not demonstrate pathological findings in the clot side middle cerebral artery was the animal in which vasospasm was not evident angiographically on Day 7 (Fig. 15).

Study A(*ii*) - Pharmacological Studies of Vessels in Spasm

Responses of clot side and non-clot side arteries to 5-hydroxytryptamine, norepinephrine, and potassium chloride, by treatment group, are shown in Figures 16 to 19. In each case there was a significant reduction in the response of the clot side middle cerebral artery relative to the non-clot side artery. The maximal responses to 5-hydroxytryptamine, norepinephrine, and potassium chloride did not differ significantly in either the clot or the

TABLE IV

Serum Nimodipine Assays by
High Performance Liquid Chromatography

Treatment Group	Monkey No.	Serum Level (ug/l)	Mean (ug/l)
Placebo	08	ND†	
	19	--‡	
	21	ND	
	29	ND	
	30	--	
	35	ND	ND
Nimodipine 3 mg/kg	10	12	
	24	10	
	31	24	
	32	ND	
	33	21	
	39	--	17 ± 7
Nimodipine 6 mg/kg	04	13	
	07	5	
	12	ND	
	20	27	
	25	10	
	26	20	15 ± 9
Nimodipine 12 mg/kg	09	14	
	14	--	
	28	99	
	34	ND	
	36	83	
	37	12	52 ± 45

†ND = none detected

‡-- = not done

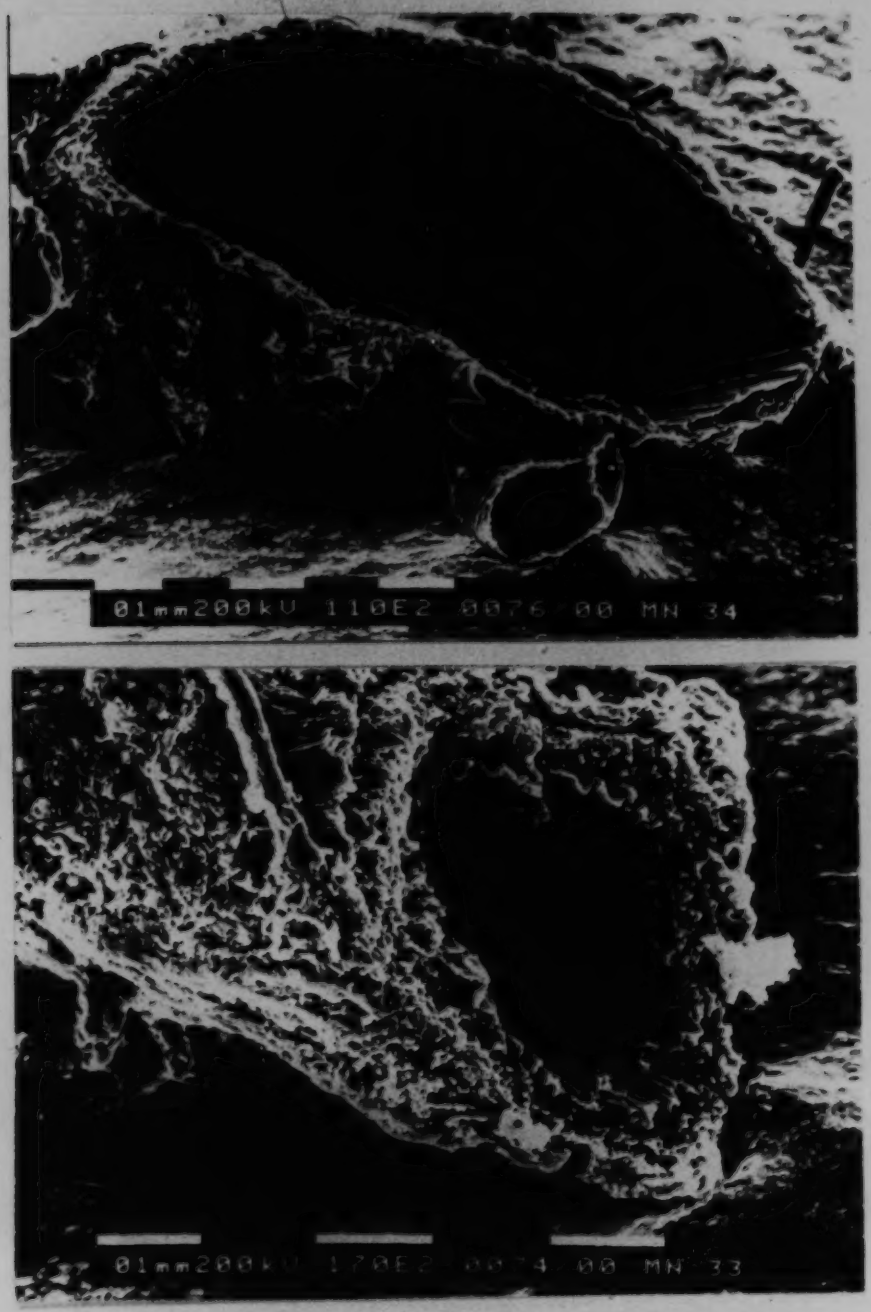


Fig. 12. Scanning electron micrographs of the middle cerebral arteries (MCAs) of a monkey from the placebo treatment group. *Top*, left MCA (non-clot side) (×110) demonstrating a mild degree of spasm. *Bottom*, right MCA (clot side) (×170) showing marked vasoconstriction.

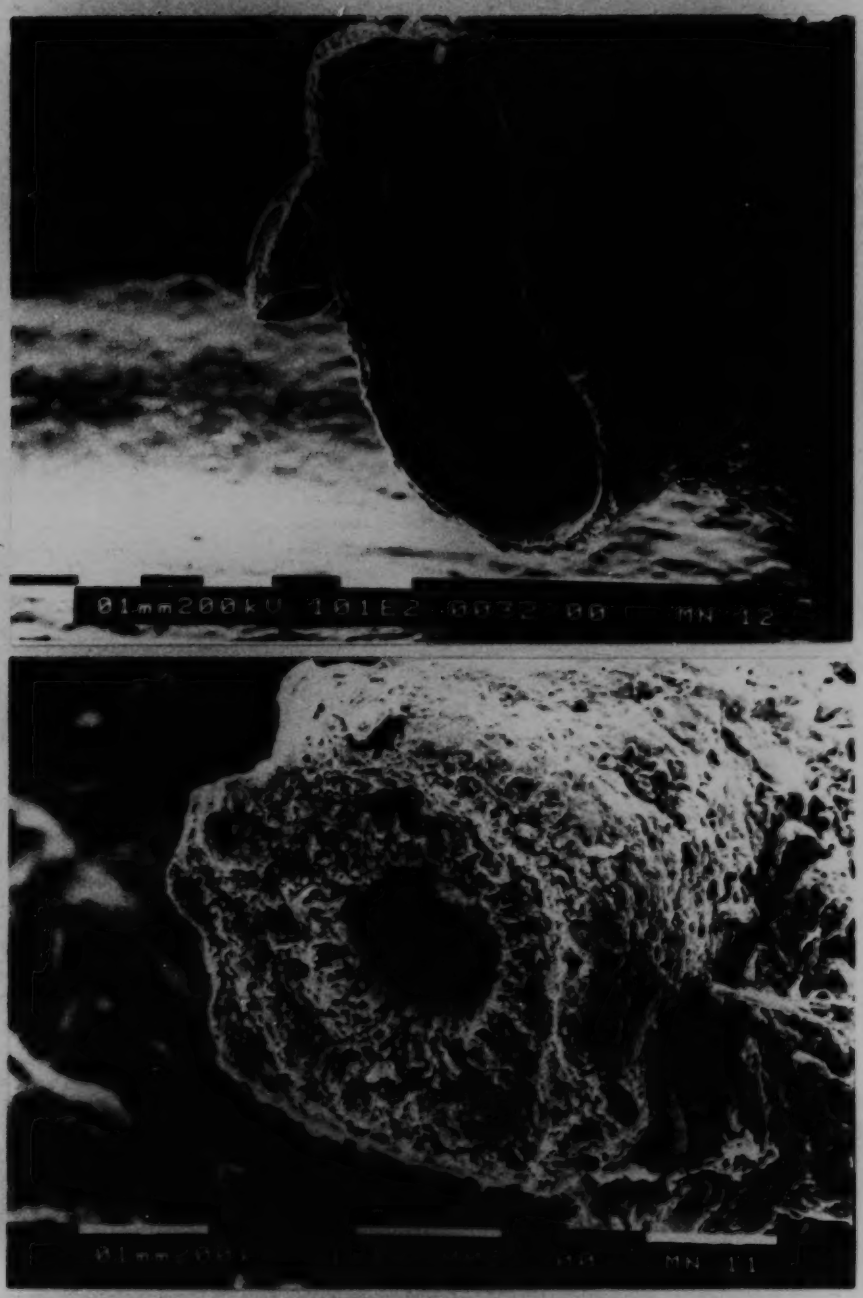


Fig. 13. Scanning electron micrographs of the middle cerebral arteries (MCAs) of a monkey treated with nimodipine, 6 mg/kg p.o. q8h. *Top*, left MCA;(non-clot side) (×101). *Bottom*, right MCA (clot side) (×212) demonstrating severe vasospasm. Note the relative magnifications which further emphasize the degree of constriction present.

Fig. 14. Representative transmission electron micrographs. *Top*, normal left middle cerebral artery (non-clot side). *Bottom*, right middle cerebral artery (clot side) demonstrating marked convolutions of the intima and media.

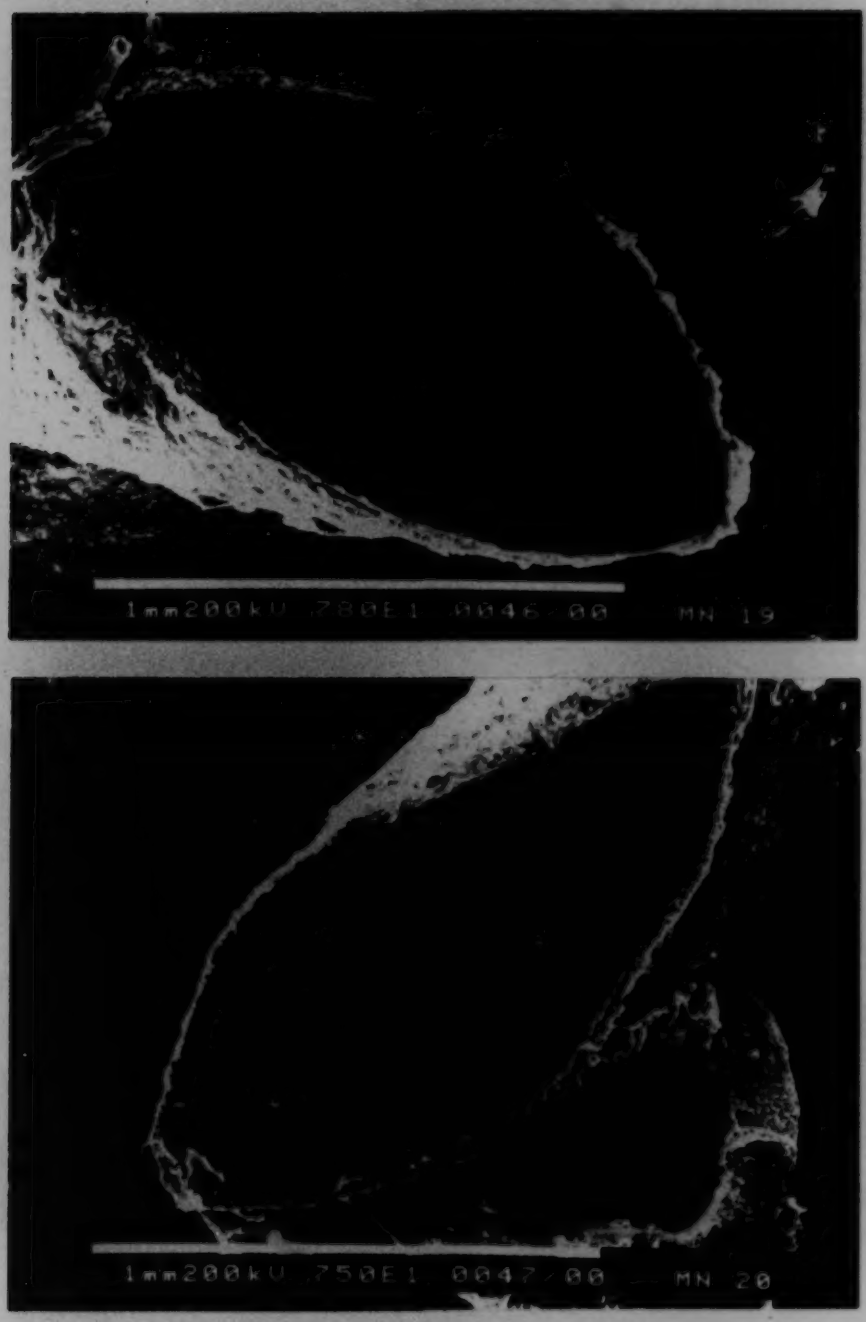


Fig. 15. Scanning electron micrographs of the middle cerebral arteries (MCAs) of the monkey in the nimodipine, 6 mg/kg p.o. q8h treatment group which did not develop angiographic vasospasm. *Top*, left MCA (non-clot side) (×78). *Bottom*, right MCA (clot side) (×75). Note the absence of any evidence of vasospasm.

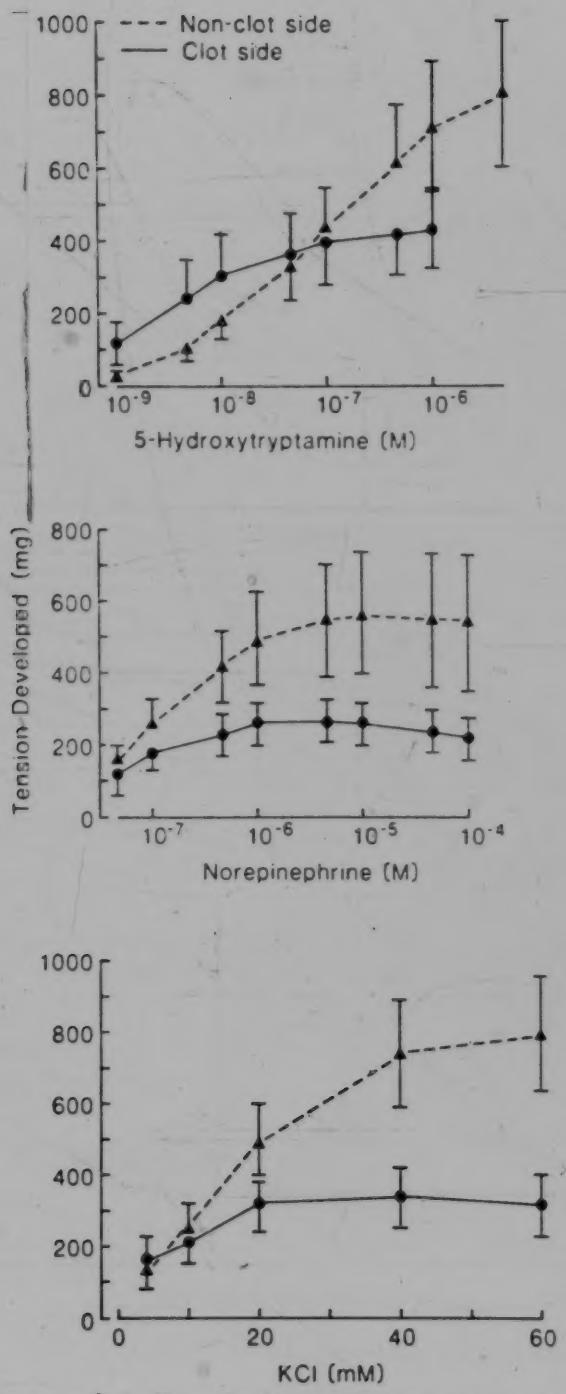


Fig. 16. Response of isolated rings of clot side and non-clot side middle cerebral arteries of the placebo treatment group to various agents. The concentration of 5-hydroxytryptamine, norepinephrine, and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. Bars represent standard errors.

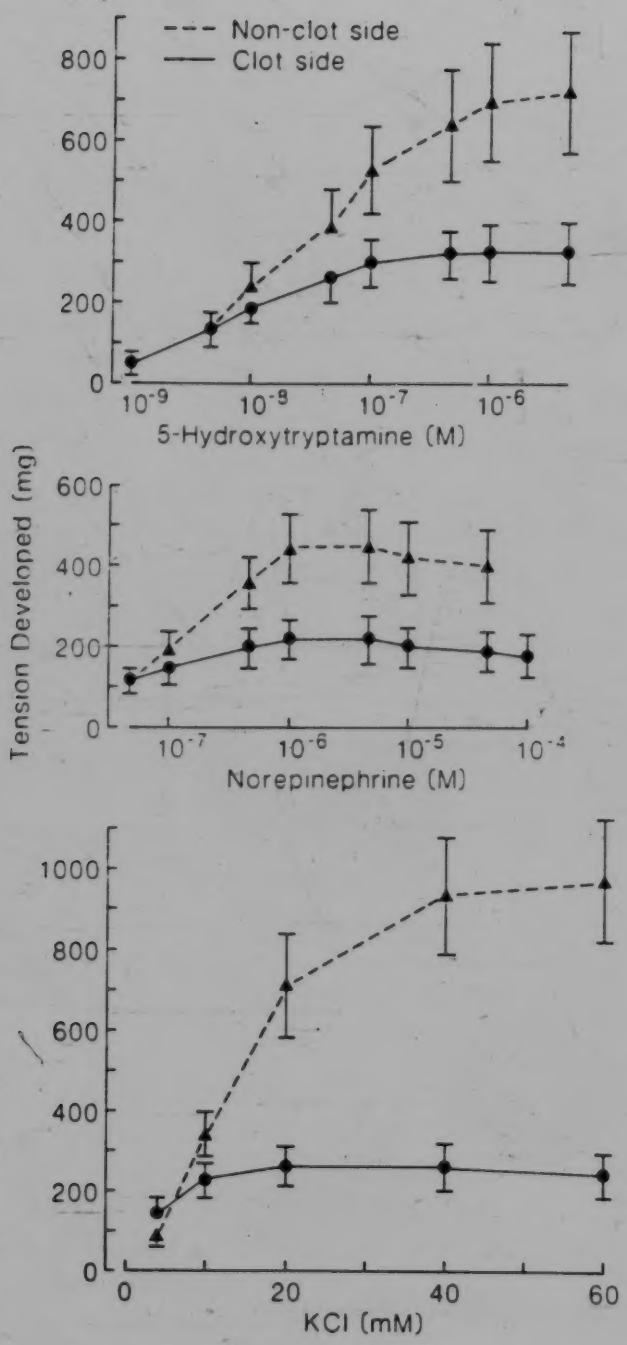


Fig. 17. Response of isolated rings of clot side and non-clot side middle cerebral arteries of the nimodipine, 3 mg/kg p.o. q8h, treatment group to various agents. The concentration of 5-hydroxytryptamine, norepinephrine and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. Bars represent standard errors.

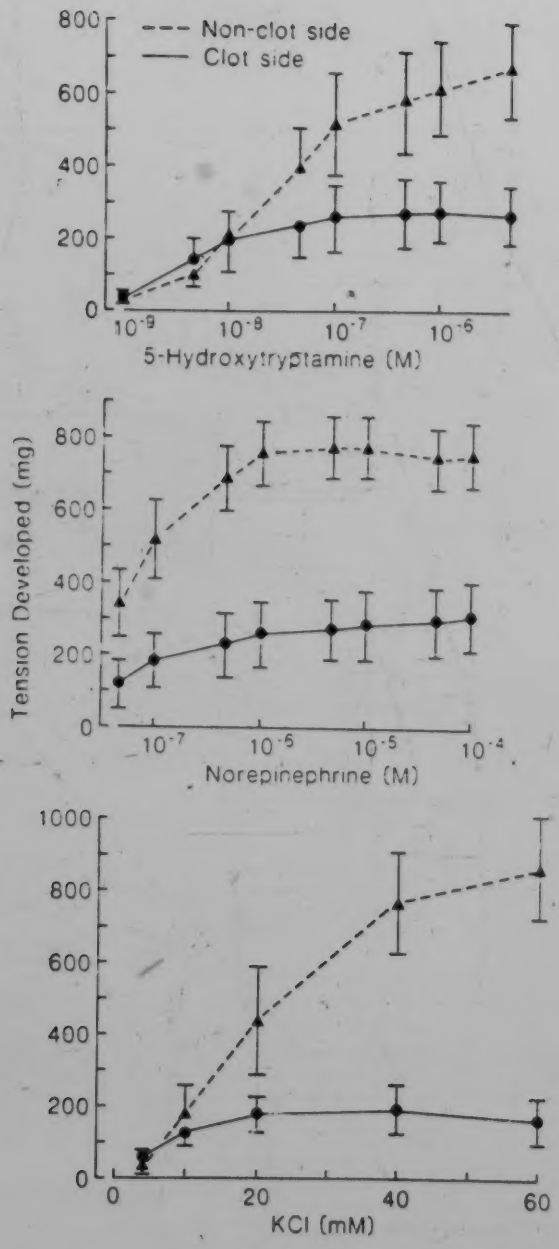


Fig. 18. Response of isolated rings of clot side and non-clot side middle cerebral arteries of the nimodipine, 6 mg/kg p.o. q8h, treatment group to various agents. The concentration of 5-hydroxytryptamine, norepinephrine and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. Bars represent standard errors.

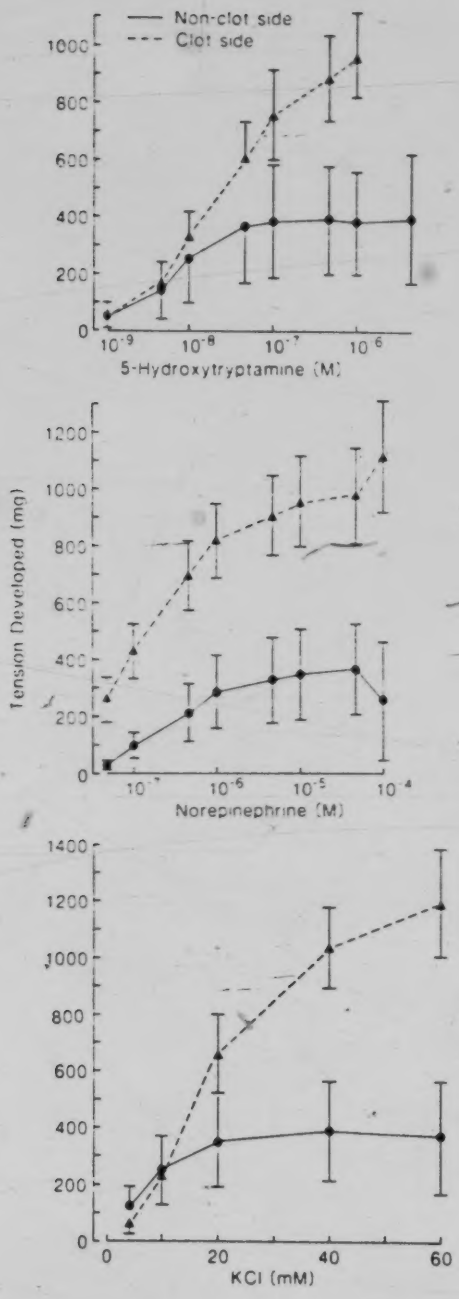


Fig. 19. Response of isolated rings of clot side and non-clot side middle cerebral arteries of the nimodipine, 12 mg/kg p.o. q8h treatment group to various agents. The concentration of 5-hydroxytryptamine, norepinephrine and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. Bars represent standard errors.

non-clot side. In arteries from the clot side, the contractility was not influenced by treatment with nimodipine at any dose tested, regardless of the contractile agent examined (Fig. 20). In the non-clot side arteries, there was a significantly enhanced reactivity of vessels from animals that had received the largest dose of nimodipine, whereas arteries from all other animals did not differ significantly. This enhanced reactivity occurred for each agonist tested (Fig. 21).

Monkey #36, which in study A(*i*) had developed the delayed ischemic deficit had the least reactive arteries to any of the agonists tested of any of the monkeys (Fig. 22).

Study A(*iii*) - *In Vitro* Affect of Nimodipine

Nimodipine is an extremely effective agent in inhibiting the contraction caused by doses of potassium chloride up to 40 mM or, in the case of arteries from the monkey, up to 60 mM. Concentrations of 10^{-8}M and higher effectively abolish the potassium-induced contractions in all species tested (Fig. 23). There was no significant difference between the species in the sensitivity to nimodipine of the potassium chloride response. In the case of 5-hydroxytryptamine-induced contractions, arteries from the monkey were much less sensitive to nimodipine (Fig. 24). At a concentration of 10^{-8}M nimodipine the response of arteries from the dog was reduced to about one third of the control value while human arteries are even more sensitive. At this concentration there was no significant attenuation by nimodipine of the response to 5-hydroxytryptamine

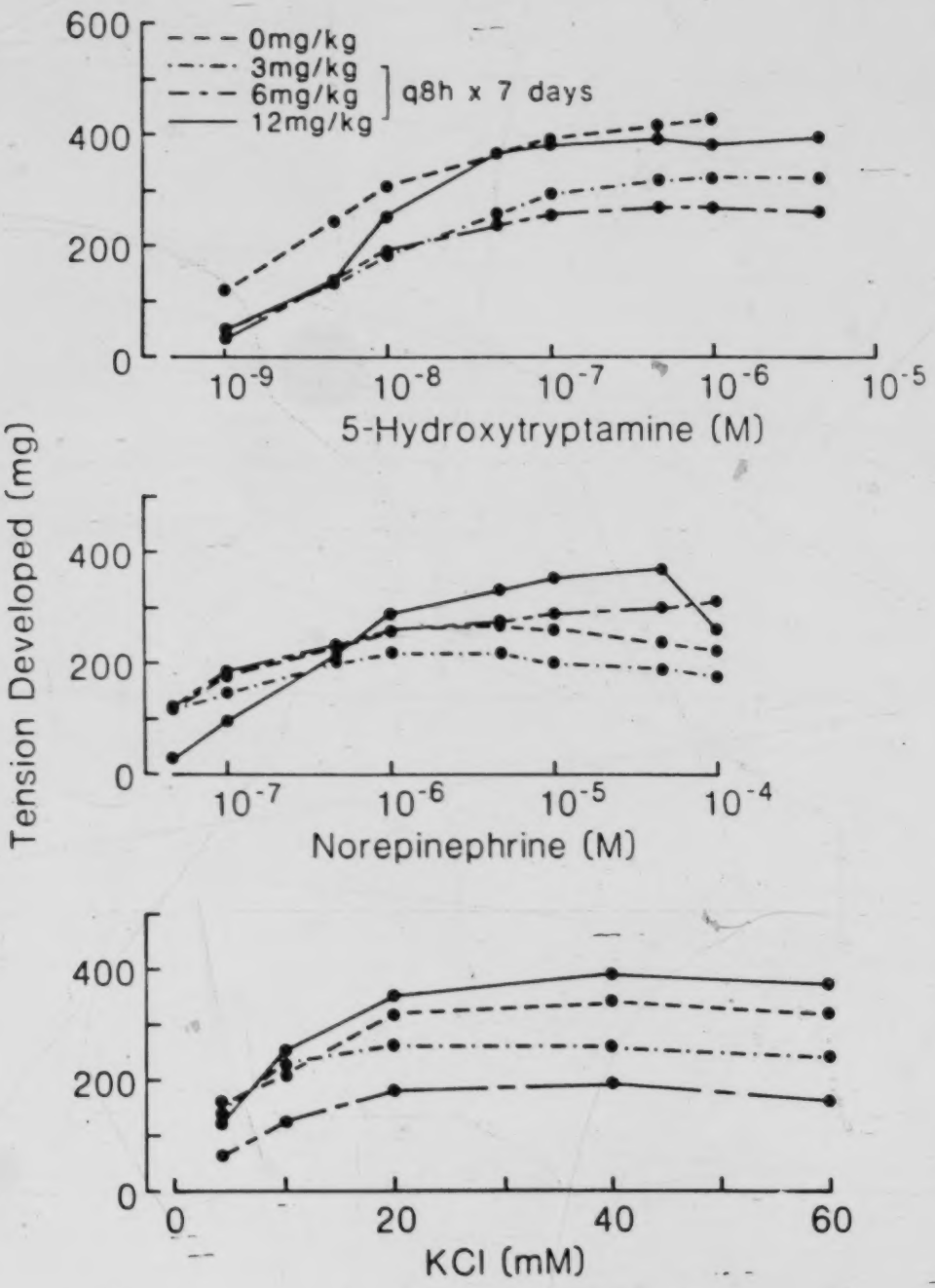


Fig. 20. Response of isolated rings of middle cerebral artery to various agents. The concentration of 5-hydroxytryptamine, norepinephrine, and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. The data in this figure were obtained from arteries in vasospasm (clot side).

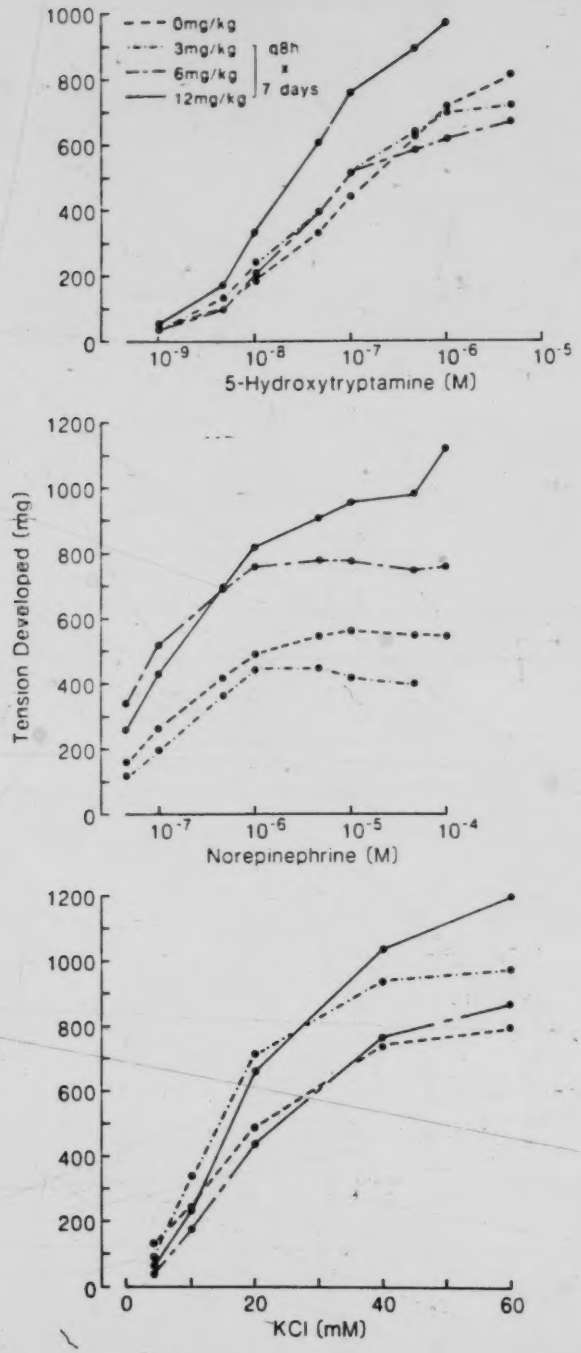


Fig. 21. Response of isolated rings of middle cerebral artery to various agents. The concentration of 5-hydroxytryptamine, norepinephrine, and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*. The data in this figure were taken from nonspastic arteries (non-clot side).

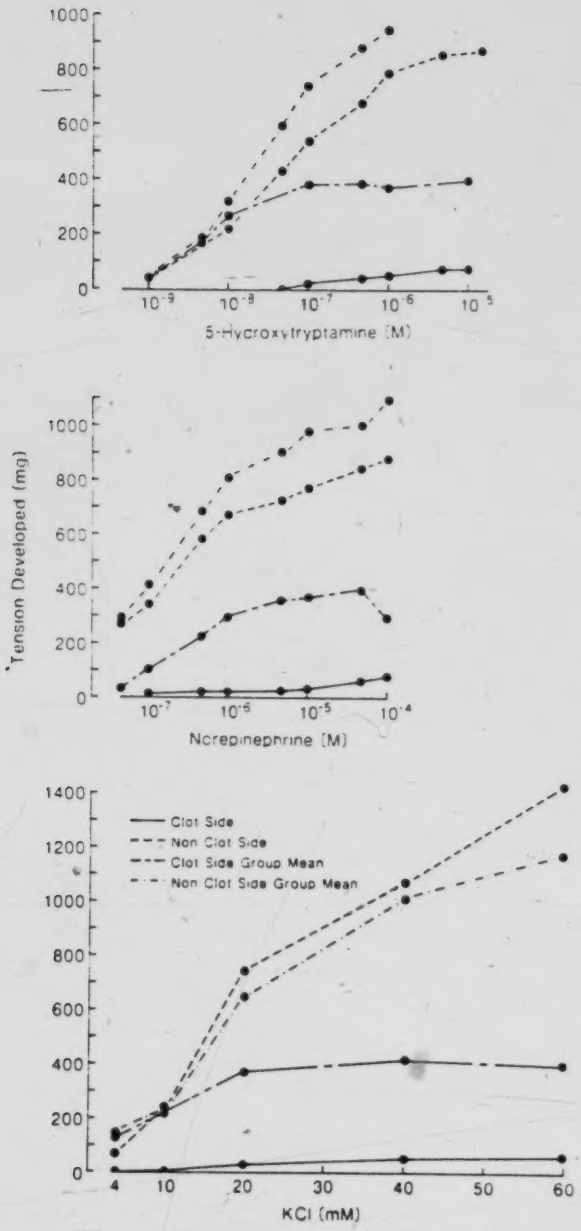


Fig. 22. Response of isolated rings of middle cerebral artery from Monkey No. 36 to various agents compared to nimodipine, 12 mg/kg p.o. q8h group means. The concentration of 5-hydroxytryptamine, norepinephrine and potassium chloride is shown as the *abscissa*; the contraction in grams is shown as the *ordinate*.

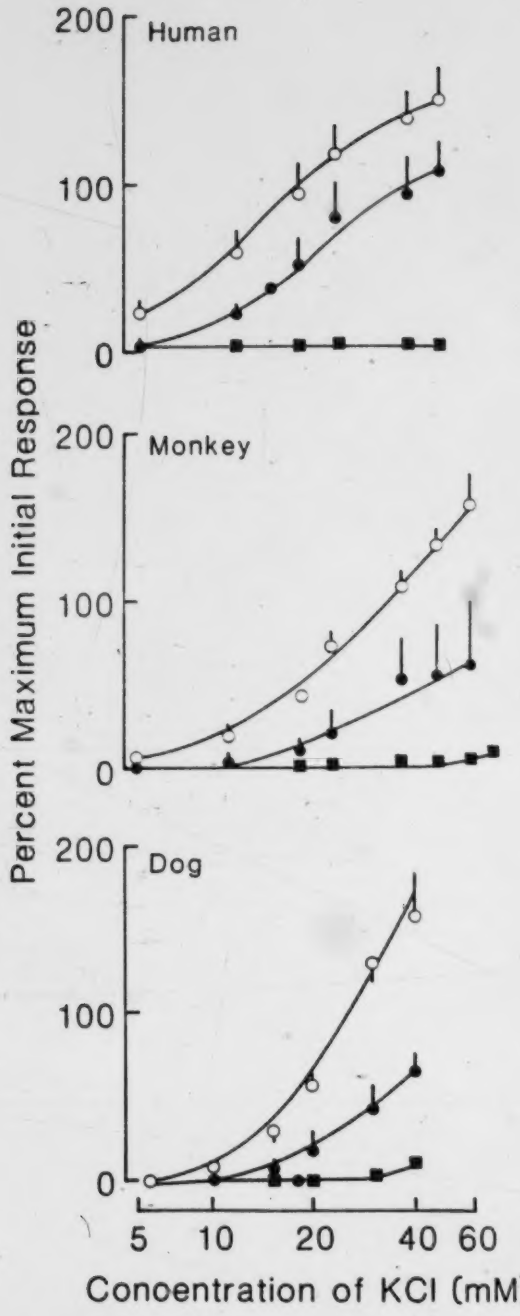


Fig. 23. Effects of different concentrations of nimodipine on the responses to potassium chloride of middle cerebral arteries of man, monkey, and dog. Logarithm of molar concentration of potassium chloride (KCl) as *abscissa*; percent maximum initial response as *ordinate*. Bars represent standard errors. ○, time control; ●, nimodipine 10^{-9} M; ■, nimodipine 10^{-8} M.

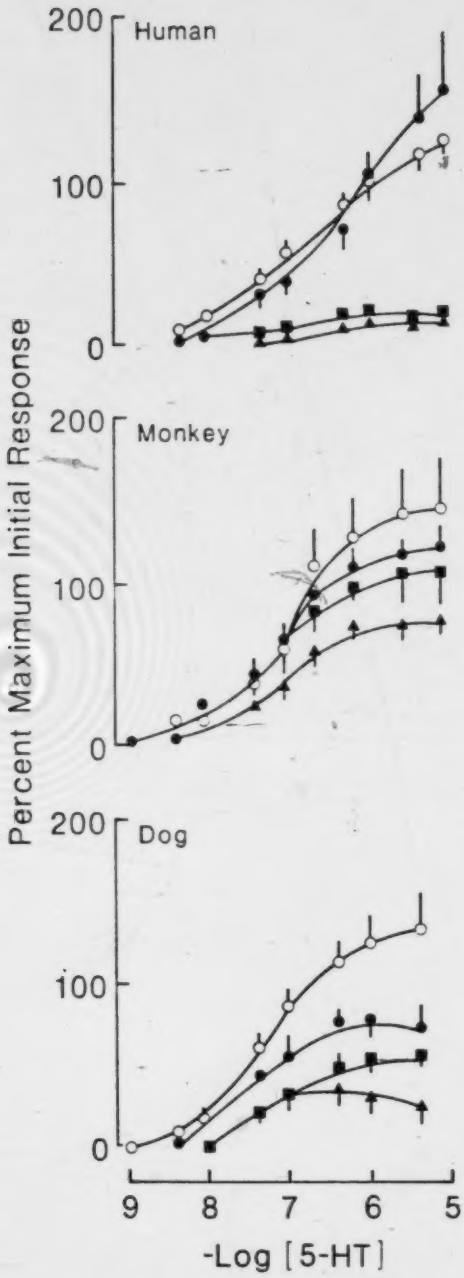


Fig. 24. Effects of different concentrations of nimodipine on the responses to 5-hydroxytryptamine of middle cerebral arteries of man, monkey, and dog. Logarithm of molar concentration of 5-hydroxytryptamine (5-HT) as *abscissa*; percent maximum initial response as *ordinate*. Bars represent standard errors. O, time control; ●, nimodipine 10^{-9} M; ■, nimodipine 10^{-8} M, ▲, nimodipine 10^{-7} M.

in the monkey. Similar results were observed in the case of norepinephrine-induced responses (Fig. 25). Low doses of nimodipine easily inhibited the response of human and dog arteries to norepinephrine while in the monkey, the responses were diminished by about 50% using 10^{-9}M nimodipine. Larger doses did not seem to produce any further effect. In the case of prostaglandin $F_{2\alpha}$ there was much less attenuation of the response in the species tested (Fig. 26). It actually appeared that in monkey and man, low doses of nimodipine enhanced the response, but this did not reach statistical significance. The responses to hemoglobin were varied. No significant effects of nimodipine even up to concentrations of 10^{-7}M could be determined (Fig. 27).

Study A(iv) - Intravenous Nimodipine and Cerebral Blood Flow

Changes in $PaCO_2$ values affected changes in measured cerebral blood flow at a rate of approximately 3% per mm Hg change in $PaCO_2$.

Continuous intravenous infusions of placebo at a fixed $\overline{PaCO_2}$ of 40 mm Hg effected no changes in cerebral blood flow.

Continuous intravenous infusions of nimodipine 2 μg/kg/min caused detectable increases in cerebral blood flow at the first post-infusion measurement time of 15 minutes. Cerebral blood flow peaked at 22% increase over basal flows at 1 hour after starting the infusion. No further increase was detected over the next 45 minutes. There was no statistically significant effect on mean arterial blood pressure or heart rate (Fig. 28).

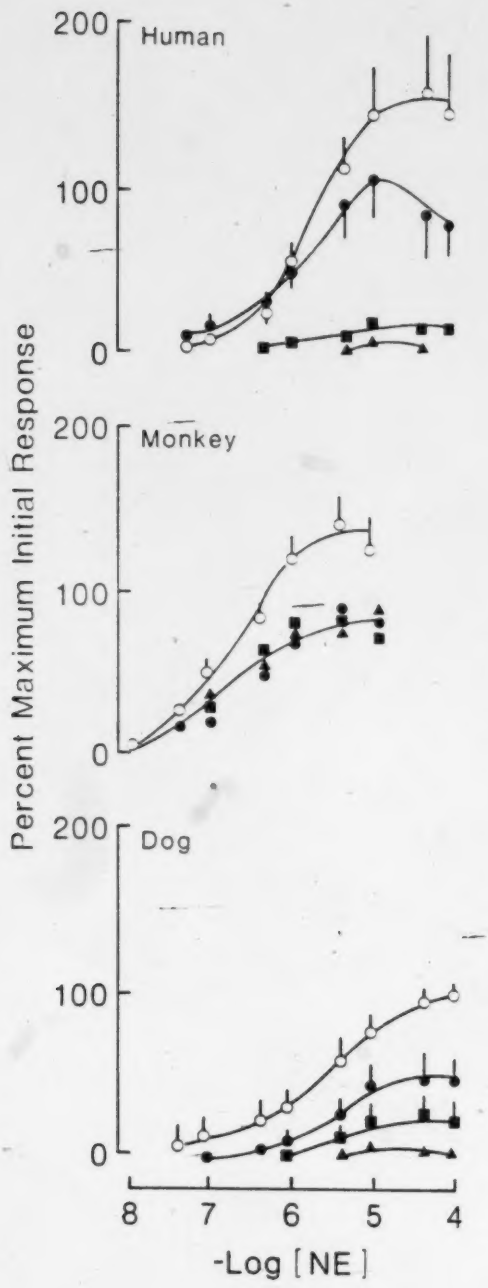


Fig. 25. Effects of different concentrations of nimodipine on the responses to norepinephrine of middle cerebral arteries of man, monkey, and dog. Logarithm of molar concentration of norepinephrine (NE) as *abscissa*; percent maximum initial response as *ordinate*. Bars represent standard errors. ○, time control; ●, nimodipine 10^{-9} M; ■, nimodipine 10^{-8} M; ▲, nimodipine 10^{-7} M.

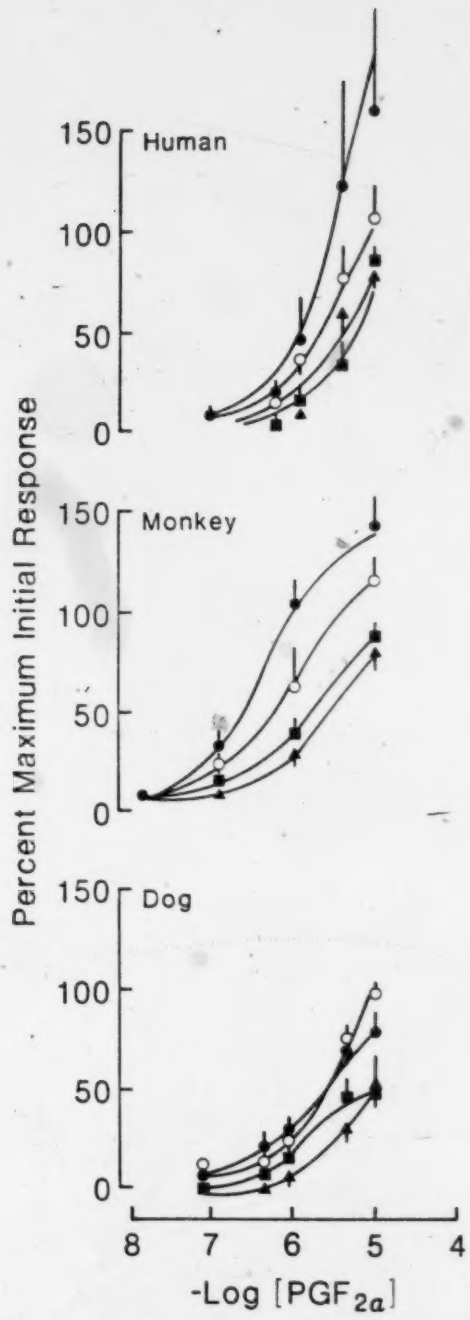


Fig. 26. Effects of different concentrations of nimodipine on the responses to prostaglandin $F_{2\alpha}$ of middle cerebral arteries of man, monkey, and dog. Logarithm of molar concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as *abscissa*; percent maximum initial response as *ordinate*. Bars represent standard errors. ○, time control; ●, nimodipine 10^{-9} M; ■, nimodipine 10^{-8} M; ▲, nimodipine 10^{-7} M.

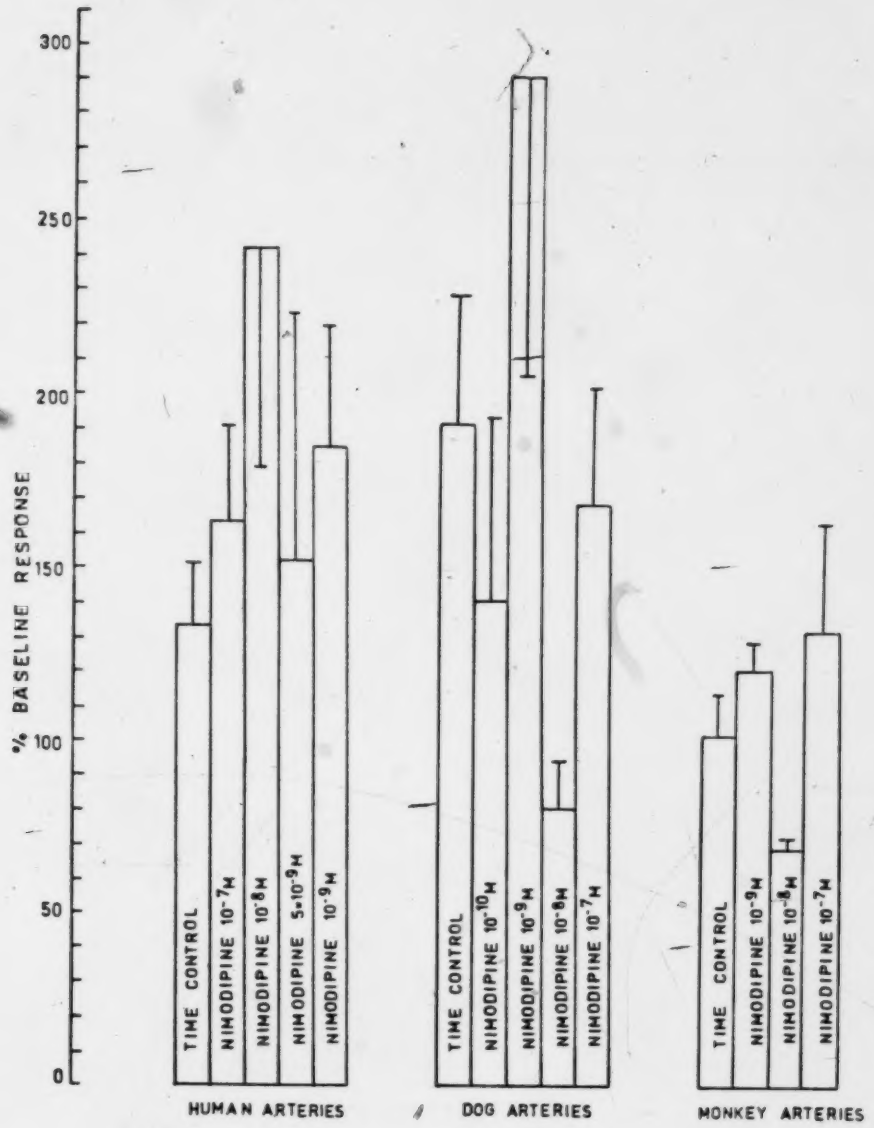


Fig. 27. Effects of different concentrations of nimodipine on the responses to hemoglobin (10^{-4} M) of middle cerebral arteries of man, monkey, and dog. Percentage initial response to 10^{-4} M hemoglobin as *ordinate*. Bars represent standard errors.

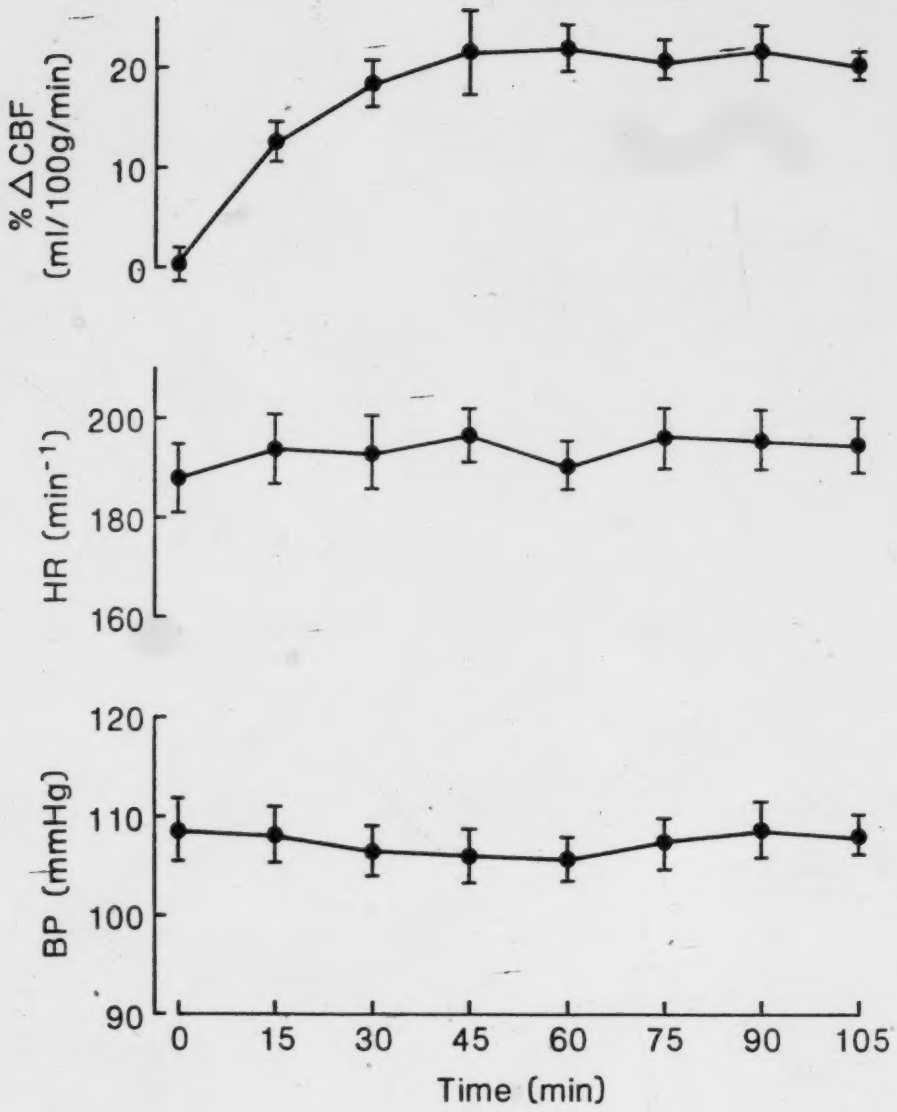


Fig. 28. Cerebral blood flow (CBF), heart rate (HR), and arterial blood pressure (BP) responses of the cynomolgus monkey to intravenous infusion of nimodipine, 2 μg/kg/min. Bars represent standard errors. n=6.

Continuous infusions of nimodipine 5 μg/kg/min caused an increase in cerebral blood flow of 34% over control flows which peaked at 45 minutes after the start of the infusion. There was no further increase over the next hour. Mean arterial blood pressure fell and heart rate increased over the first 30 minutes but both recovered to near baseline values by one hour (Fig. 29).

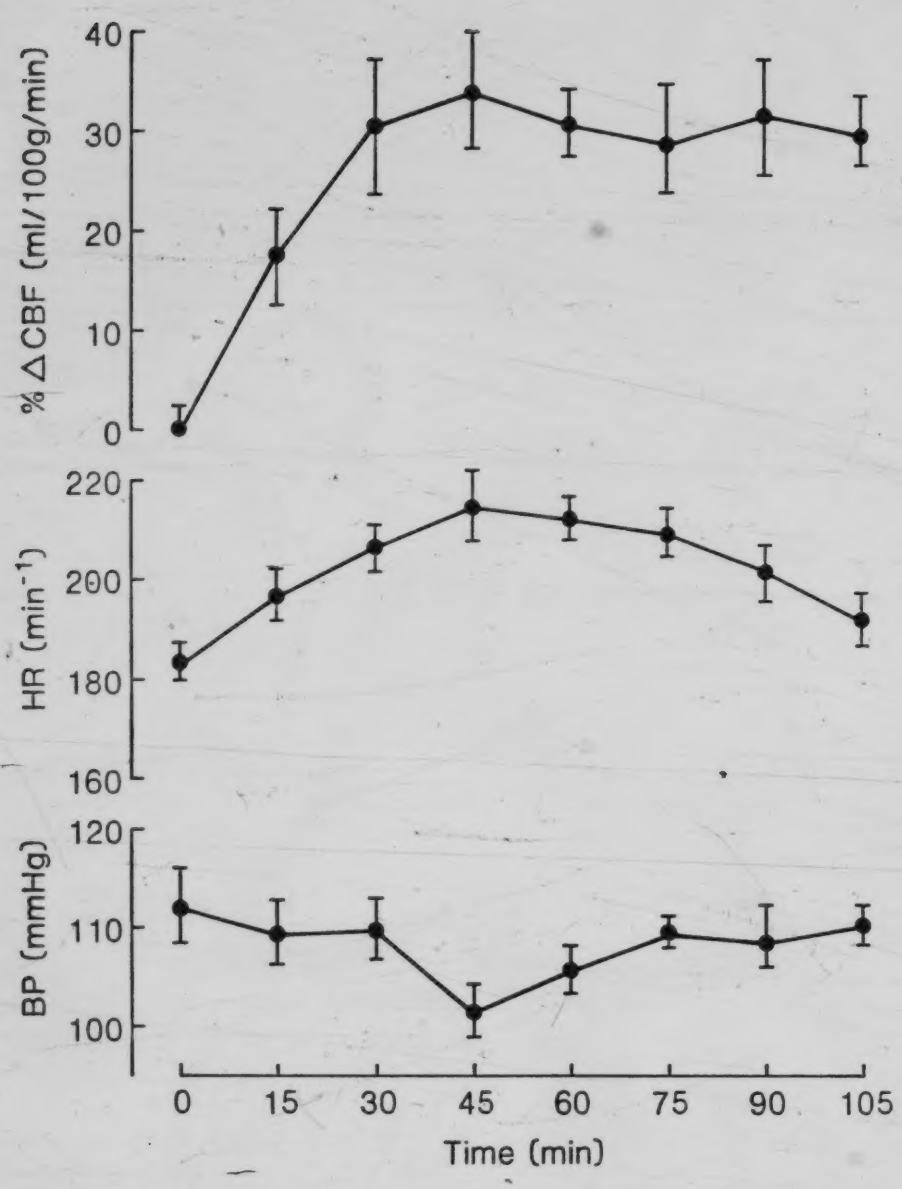


Fig. 29. Cerebral blood flow (CBF), heart rate (HR), and arterial blood pressure (BP) responses of the cynomolgus monkey to intravenous infusion of nimodipine, 5 μg/kg/min. Bars represent standard errors. n=6.

Study B - Early Clot Removal and Cerebral Vasospasm

Study B(*i*) - Clinical, Radiological, and Pathological Findings

Twenty-three of the 24 animals started in the study survived to the time of completion. One animal in the clot group (No. 100) developed a progressive left hemiparesis on Day 5. On Day 7, the right hind limb was also paretic. Another animal in the clot group (No. 125) died on Day 4 from a massive cerebellar infarction. One animal in the clot group (No. 111) suffered an inadvertent intramuscular injection of pentobarbital when an intravenous line went interstitial while the animal was in the magnetic resonance imaging cylinder. The leg became gangrenous on Day 4 post-subarachnoid hemorrhage. The animal underwent surgery for an above knee amputation under general anaesthesia on Day 4 and survived to completion of the experiment without any adverse signs.

Statistical comparisons within each group and across the 3 groups showed no significant differences in baseline values, Day 7 values, or between baseline and Day 7 values in body weight or measured physiological indices (pH, $PaCO_2$, PaO_2, mean arterial blood pressure, heart rate). The baseline data were therefore grouped for the statistical analysis (Table V).

Comparisons between the clot group and the clot-removal group revealed no statistically significant differences in the weight of the clot placed. The difference between amount of clot placed (4.7 g) and amount of clot removed (1.5 g) on Day 1 in the clot-removal group was statistically significant ($p < 0.05$).

TABLE V

Clot Removal and Chronic Cerebral Vasospasm

Values for Measured and Observed Indices ($\bar{x}\pm se$)[†]
For Each Treatment Group

Parameter		Pre-SAH	Day 7 Post-SAH		
			Sham	Clot-Removal	Clot[‡]
No. of Monkeys		24	8	8	7
Body Weight		3.6±0.4	3.2±0.4	3.7±0.4	3.9±0.4
MABP (mm Hg)		109±8	110±3	112±6	110±10
HR (per min)		156±18	159±12	157±17	152±15
$PaCO_2$ (mm Hg)		39±1	39±1	39±1	39±1
Vessel Caliber					
ICA	1	1.52±0.12	1.41±0.22	1.51±0.17	1.27±0.25
	2	1.50±0.11	1.38±0.25	1.49±0.15	0.26±0.33
	3	1.18±0.09	1.13±0.17	1.18±0.19	0.93±0.17
	4	1.09±0.09	1.01±0.19	1.11±0.13	0.87±0.17
ACA	5	0.83±0.08	0.64±0.18	0.83±0.15	0.56±0.13
	6	0.85±0.08	0.83±0.12	0.83±0.11	0.52±0.14
MCA	7	1.01±0.07	0.95±0.15	0.97±0.12	0.51±0.11
	8	0.96±0.07	0.87±0.20	1.03±0.18	0.50±0.22
PCA	9	0.84±0.08	0.71±0.14	0.88±0.16	0.72±0.15
	10	0.80±0.08	0.72±0.21	0.82±0.15	0.81±0.18
Size of Hematoma					
Placed (g)		n/a*	n/a	4.7±0.4	5.3±0.5
Removed (g)		n/a	n/a	1.5±0.2	n/a

[†]MABP, mean arterial blood pressure; HR, heart rate; 1, right extradural internal carotid artery; 2, left extradural internal carotid artery; 3, right supraclinoid internal carotid artery; 4, left supraclinoid internal carotid artery; 5, right anterior cerebral artery; 6, left anterior cerebral artery; 7, right sphenoidal middle cerebral artery; 8, left sphenoidal middle cerebral artery; 9, proximal pericallosal artery; 10, distal pericallosal artery.

[‡]1 animal of the clot group died on Day 4.

*n/a = not applicable

Cerebral Vasospasm

Vasospasm was defined as a 10% or greater reduction in vessel caliber from the baseline value. Vasospasm was not present on Day 7 in the sham operated animals (Fig. 30).

Vasospasm was present in all 8 animals of the clot group on Day 7. Moderate vasospasm (31 to 50% reduction in vessel caliber) was present in two of eight (25%) animals of the clot group. Severe vasospasm (> 50% reduction in vessel caliber) was present in five of eight (62%) of the clot animals (Table VI, Fig. 31). Day 7 angiography was not available on the one animal that died on Day 4.

Mild vasospasm (10 to 30% reduction in vessel caliber) was present on Day 7 in a limited number of vessels in two of eight (25%) of the clot-removal group (Fig. 32).

The reduction in vessel caliber between baseline and Day 7 in the clot group animals was statistically significant ($p < 0.01$). The difference in vessel caliber between baseline and Day 7 in the sham group and the clot-removal group was not statistically significant ($p > 0.05$).

Intergroup comparisons of Day 7 vessel calibers revealed no statistically significant differences between the sham group and the clot-removal group.

Intergroup comparisons of Day 7 vessel calibers revealed statistically significant differences between the clot group and the sham group and between the clot group and the clot-removal group in all measured vessels except the pericallosal artery ($p < 0.05$) (Fig. 33).

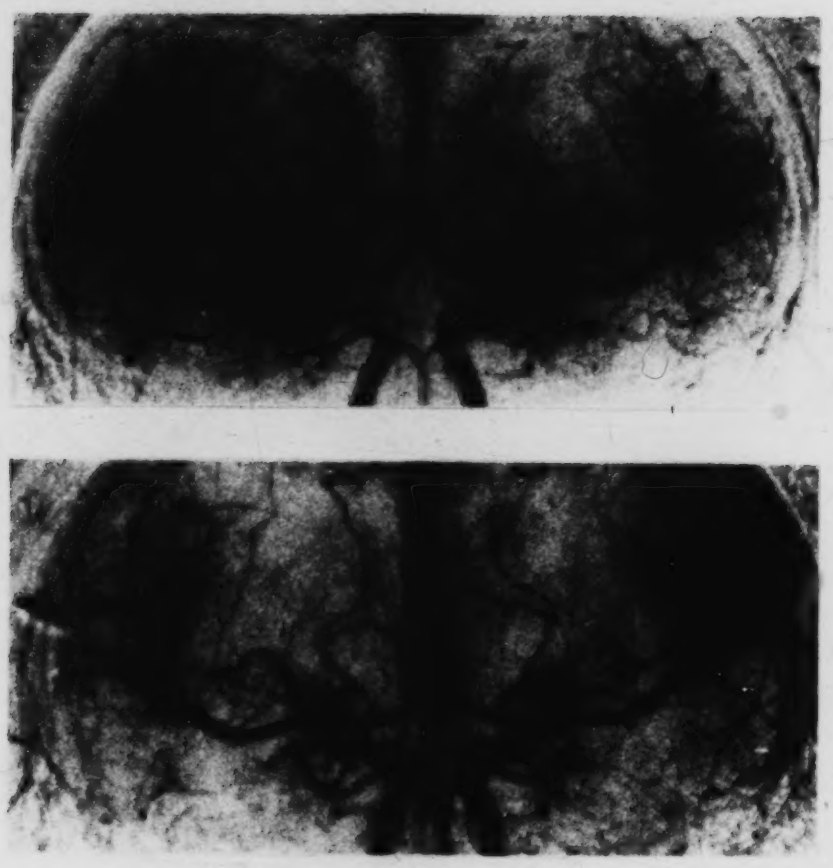


Fig. 30. Cerebral angiograms of a sham-operated animal (bilateral dissection). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage. Note the absence of any angiographic vasospasm.

TABLE VI

DEGREE OF VASOSPASM DEVELOPED BY

TREATMENT GROUP: STUDY B(*i*)

Treatment Group	Number of animals developing vasospasm		
	Mild	Moderate	Severe[†]
Sham (n=8)	0	0	0
Clot (n=7[‡])	0	2	5
Clot-removal (n=8)	2	0	0

[†]mild, 11% to 30% reduction in vessel caliber: moderate, 31% to 50% reduction in vessel caliber; severe, > 50% reduction in vessel caliber.

[‡]Day 7 angiography was not available on the animal which died on Day 4.

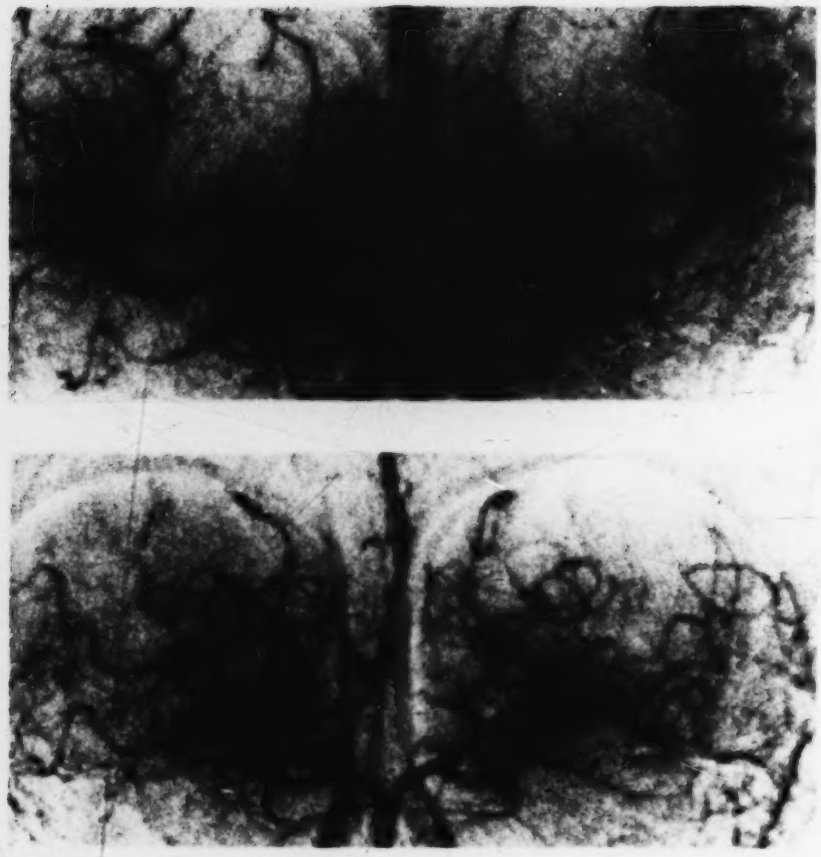


Fig. 31. Cerebral angiograms of a clot group animal (bilateral dissection and clot placement). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage. Note the marked vasospasm which extends down the extracranial internal carotid arteries.

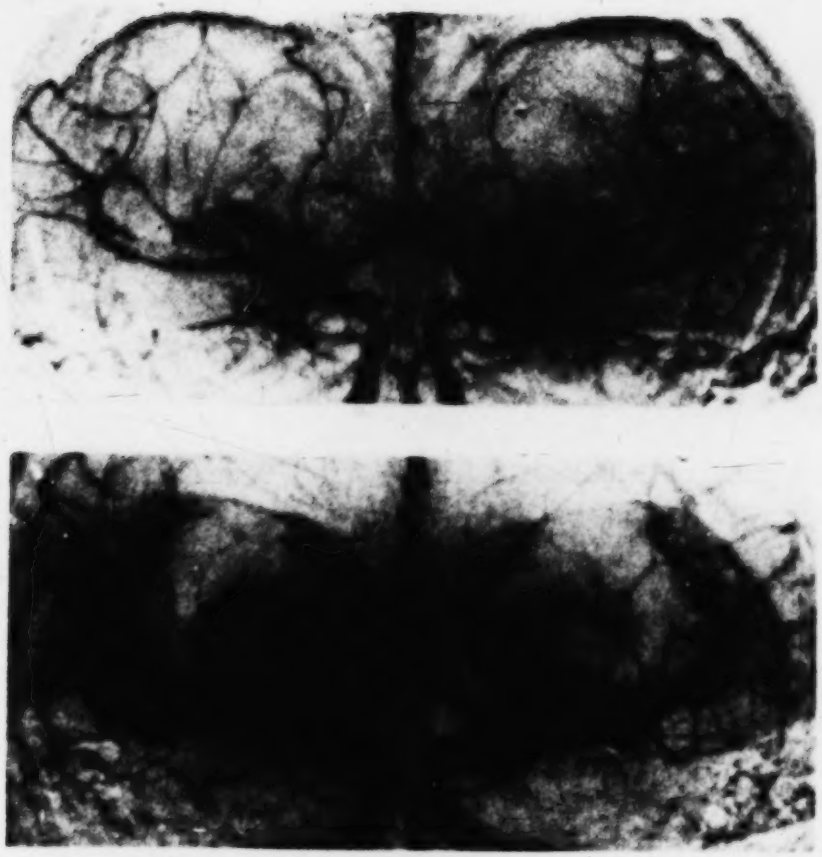


Fig. 32. Cerebral angiograms of a clot-removal group animal (bilateral dissection; clot placement; and clot removal at 24 hours). *Top*, baseline. *Bottom*, 7 days post subarachnoid hemorrhage. Note the absence of any angiographic vasospasm.

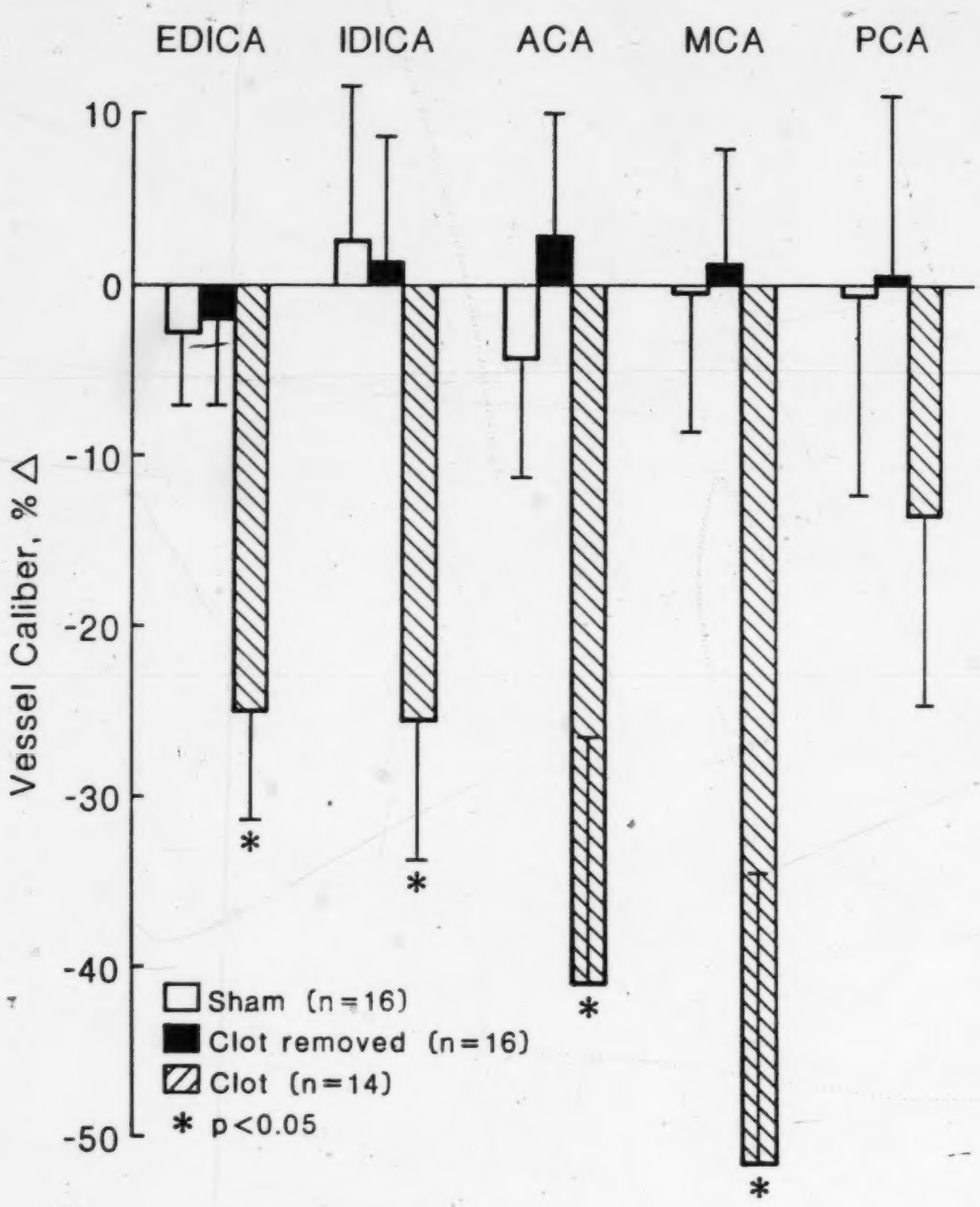


Fig. 33. Percentage change in vessel caliber from baseline of the bilateral major cerebral vessels ($\bar{x}\pm se$). EDICA, extradural internal carotid artery; IDICA, intradural internal carotid artery; ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, pericallosal artery.

Imaging

Magnetic resonance imaging defined the extent of hematoma placed during subarachnoid hemorrhage induction. Similarly, complete removal of hematoma was demonstrated by imaging on Day 1 in the clot-removal group (Fig. 34). While CT imaging produced similar results, the images were not as clear as magnetic resonance images due to a greater amount of artifact present.

Areas of infarction were clearly evident in the magnetic resonance imaging scans of monkey #100 (clot group). A large area of increased signal could be seen in the territory of the right middle cerebral artery. A smaller infarct could be seen in the left cerebral hemisphere which accounted for the mild right leg deficit detected on Day 7. The large, right-sided infarct was also detected on CT scan, although much less clearly. The smaller, left-sided infarct could not be seen on the CT (Fig. 35).

Surprisingly, a small area of increased signal return was detected in the right pallidal area of monkey #105. This was a clot-removal group animal and there had been no evidence of neurologic dysfunction. The histology and location indicated that it had occurred at the time of subarachnoid induction and was likely to have been the result of operative damage to a small perforating artery (Fig. 36).

Areas of ischemia were much more clearly delineated by magnetic resonance imaging than by CT scan.

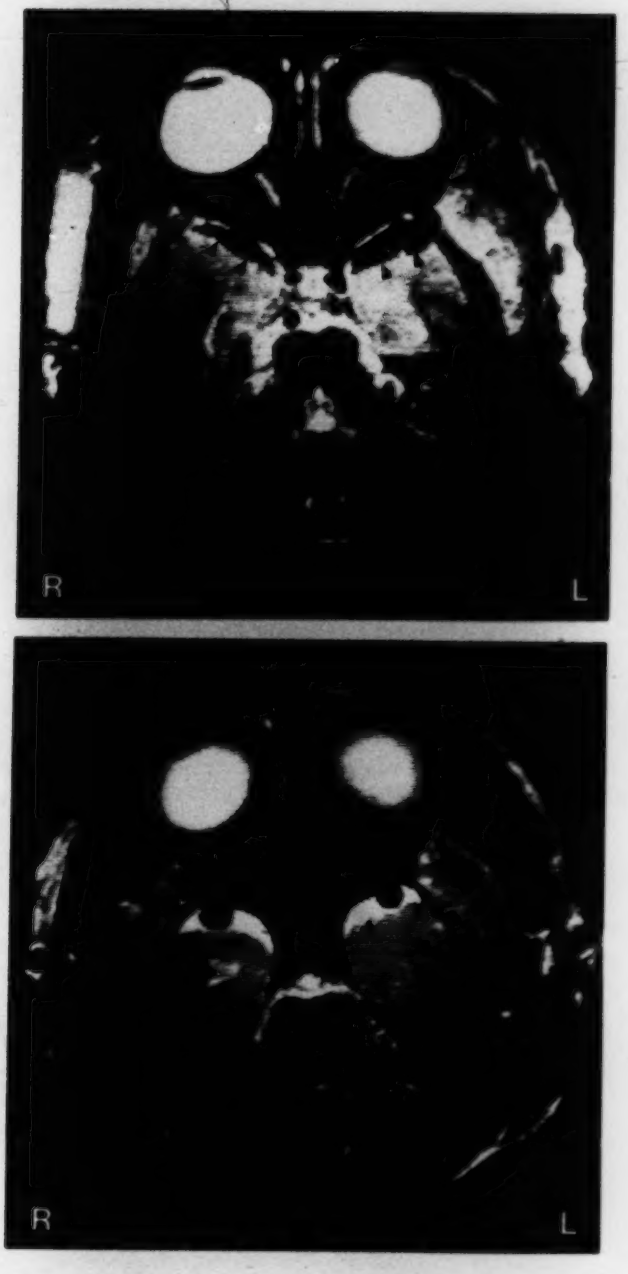


Fig. 34a. *Top*, representative magnetic resonance image (MRI) obtained immediately after subarachnoid hemorrhage induction. Blood clot is depicted by areas of low signal intensity (arrowheads). *Bottom*, MRI obtained immediately after removal of subarachnoid hematoma (24 hours post-hemorrhage). The space previously occupied by clot is now filled with cerebrospinal fluid (arrowheads) which returns a high intensity signal.

Fig. 34b. Magnetic resonance image of the same animal in Fig. 34a obtained 7 days after the subarachnoid hemorrhage. The brain has now re-expanded to fill the area left after removal of the blood clot. The high intensity signal of the cerebrospinal fluid is no longer prominent.

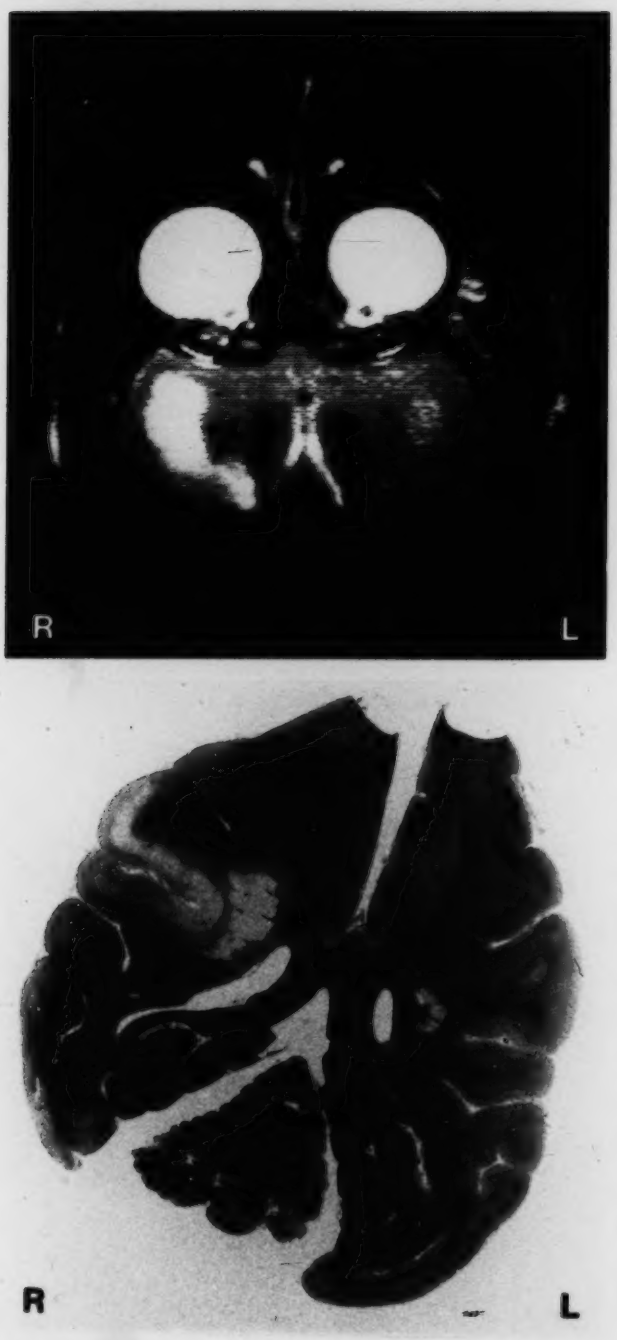
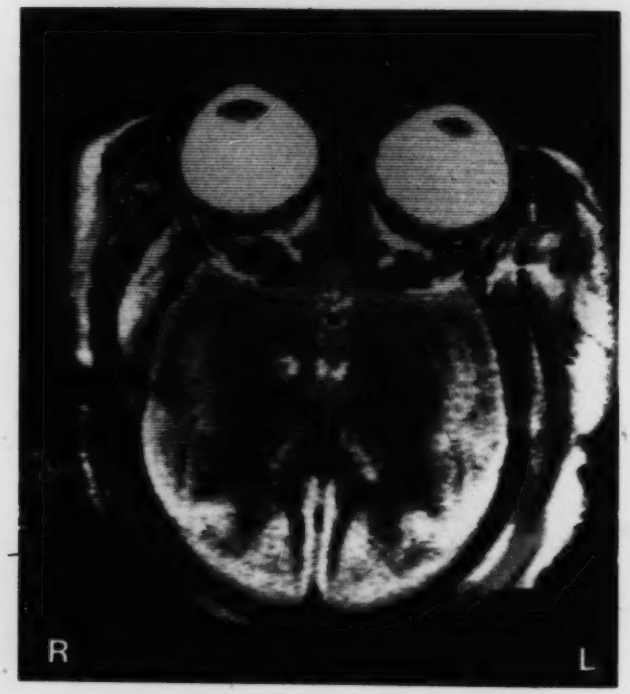


Fig. 35. *Top*, magnetic resonance image of Monkey No. 100 demonstrates the bilateral infarction which correlates with the clinical findings (arrowheads). *Bottom*, hematoxylin and eosin stained brain section which confirms the pathology (arrowheads).



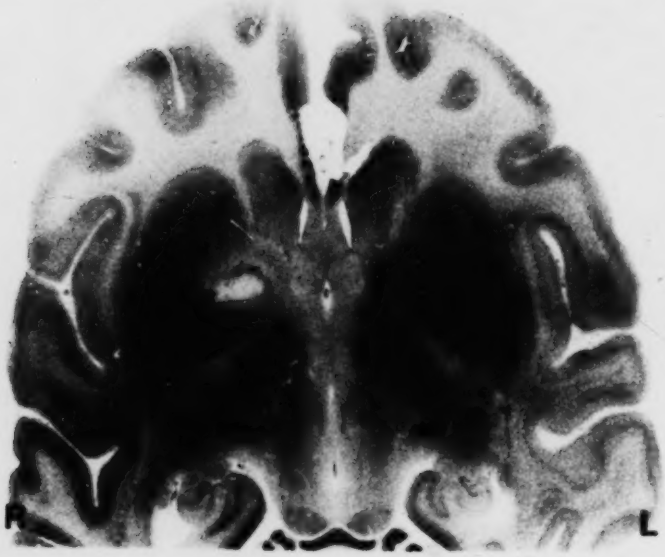


Fig. 36a. *Top*, magnetic resonance image of Monkey No. 105 which reveals a clinically unsuspected infarction of the right globus pallidus (arrowhead). *Bottom*, hematoxylin and eosin stained brain section confirms the pathology (arrowhead).

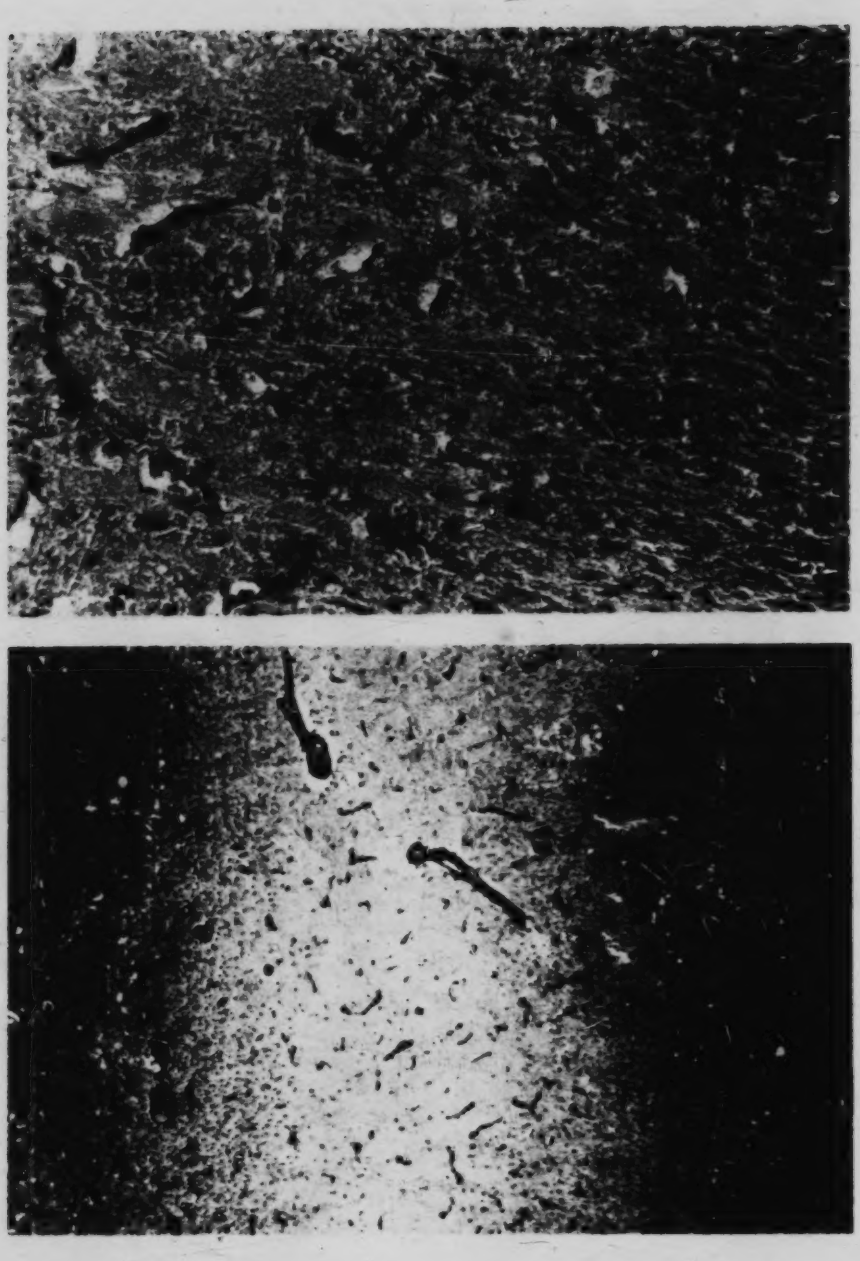


Fig. 36b. Microscopic sections of the areas of infarction depicted in Fig. 36a demonstrate a degree of histiocytic infiltration and vascular proliferation consistant with an infarction of 7 days of age. This suggests that the infarction was the result of surgical manipulation and not vasospasm.

Pathology

Pathological examination of the brain of monkey #100 showed multifocal areas of infarction, consistent histologically with a duration of approximately 3 to 4 days. The largest area of necrosis was situated in the right parietofrontal area and involved both cortex and subjacent white matter. Recent infarction unaccompanied by glial or histiocytic reaction was also present in the left caudate nucleus.

Neuropathological examination of the monkey which died on Day 4 demonstrated a large area of recent infarction within the superior cerebellar artery distribution to the right hemisphere (Fig. 37).

The brain of the monkey which had demonstrated the clinically unsuspected infarct of MRI revealed a discrete area of necrosis, 2 x 3 mm, in the right globus pallidus. The reactive cellular response in this lesion was consistent with a period of 7 days. This would indicate that the infarct occurred at the time of subarachnoid hemorrhage induction, and was not the result of vasospasm.

Study B(*ii*) - Basal Prostacyclin and Thromboxane A_2 Production

The results of the basal production level testing of prostacyclin and thromboxane A_2 as their stable metabolites 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 are given in Figures 38 and 39. Statistical analysis demonstrated no difference among the groups or the individual vessels for levels of thromboxane B_2. Levels of 6-keto-prostaglandin $F_{1\alpha}$ were found to be significantly different between the clot and clot-removal groups for the middle cerebral arteries.

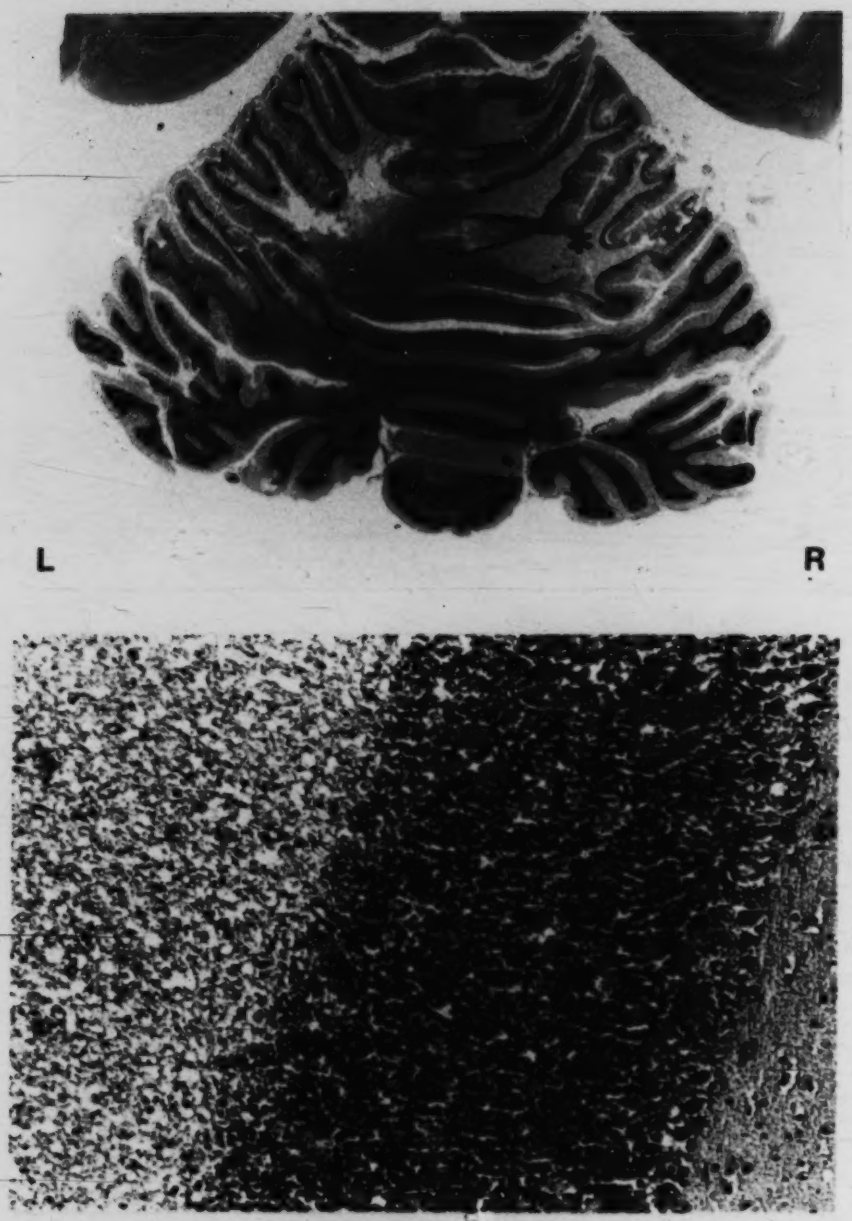


Fig. 37. *Top*, pathological section of the cerebellum of Monkey No. 125 revealed a large cerebellar infarct in the territory of the right superior cerebellar artery (*). *Bottom*, enlargement of the margin of the infarct fails to reveal any histiocytic reaction, suggesting that death occurred soon after the infarction.

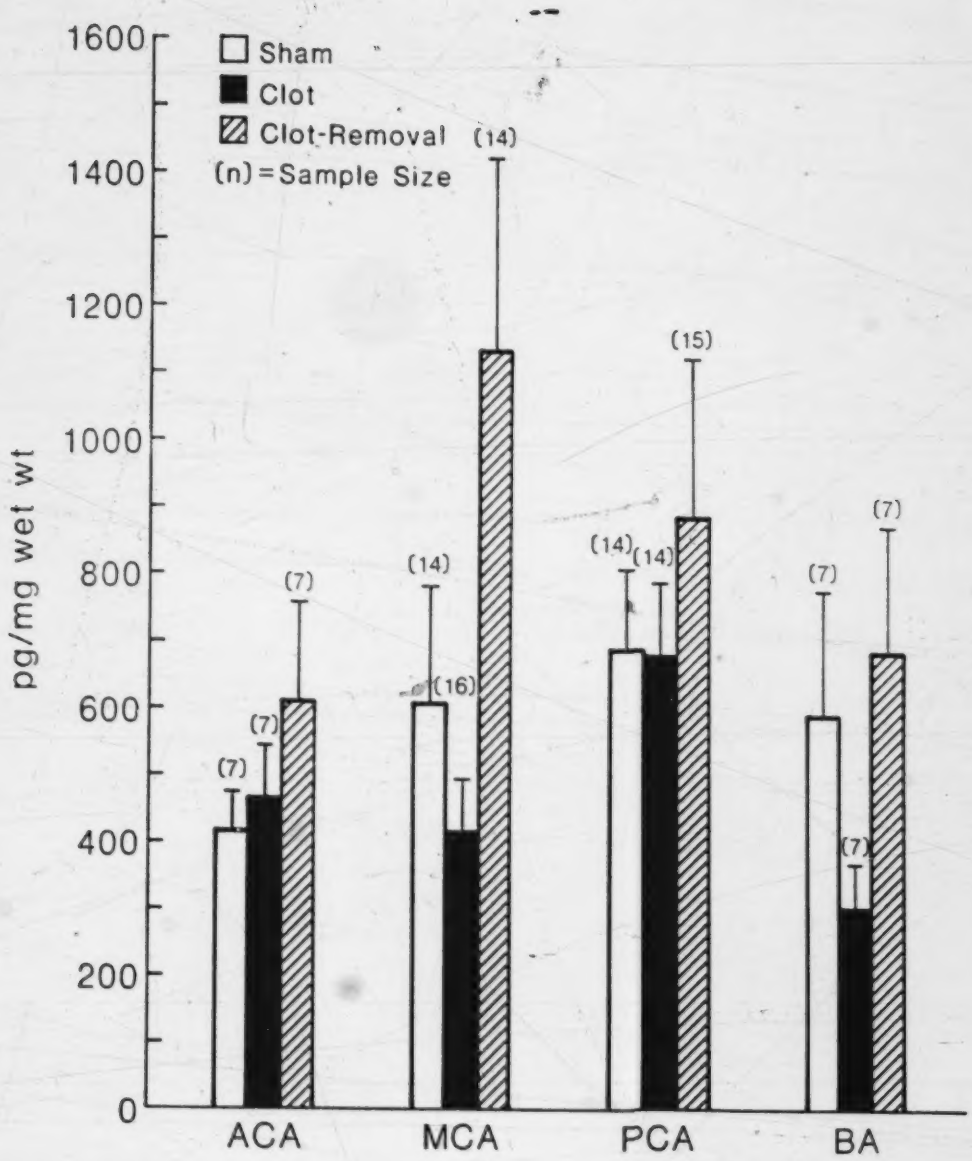


Fig. 38. Basal levels of 6-keto-prostaglandin $F_{1\alpha}$ by artery and by treatment group as determined by radioimmunoassay. ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; BA, basilar artery. Bars represent standard errors.

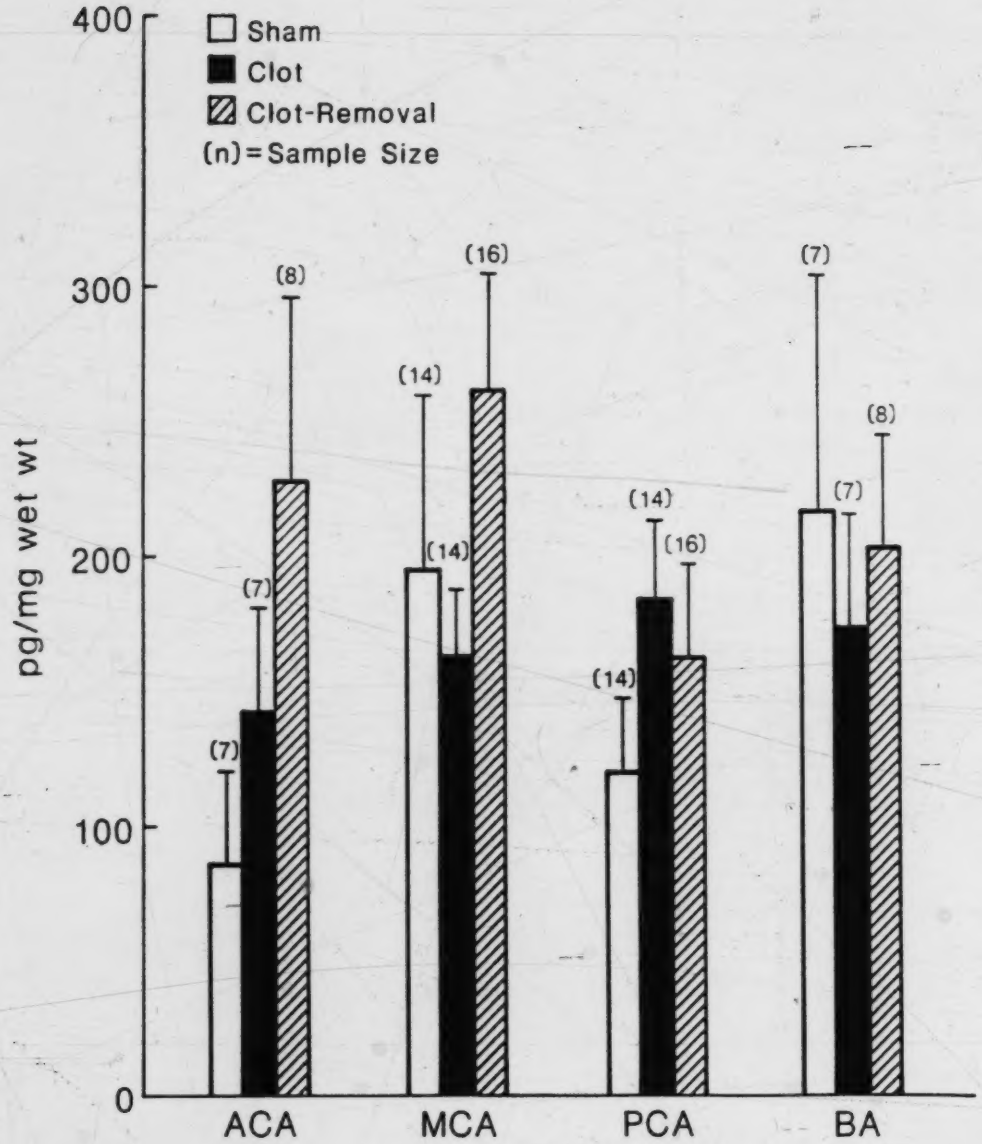


Fig. 39. Basal levels of thromboxane B_2 by artery and by treatment group as determined by radioimmunoassay. ACA, anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; BA, basilar artery. Bars represent standard errors.

Combining all arteries in each group and comparing against the other groups raised the statistical significant of clot versus clot-removal to $p < 0.01$.

No differences were detected between the sham and clot groups or between the sham and clot-removal groups.

CHAPTER FOUR: DISCUSSION

Previous study of this model in this laboratory indicated that it reliably reproduced chronic cerebral vasospasm that closely paralleled the human clinical condition after the induction of a subarachnoid hemorrhage (39,204). Furthermore, to our knowledge, it is the only model that has produced pathologically proven delayed ischemic deficits due to vasospasm. It is necessary to consider whether the surgical manipulations necessary for the induction of the subarachnoid hemorrhage may be a factor in the resulting vasospasm and delayed ischemic deficit. Studies by other investigators using both cat (93, 168) and primate (94, 133, 167, 194) models of cerebral ischemia and infarction with middle cerebral artery manipulation have shown that, when neurological deficits occur, they do so immediately after the arterial manipulation. In one study with squirrel monkeys (167), the middle cerebral artery was occluded with a Mayfield clip. Those animals in which the artery was permanently occluded uniformly developed a dense hemiplegia immediately after the occlusion or they died. Those animals undergoing temporary occlusion (6 hours) either developed a deficit immediately or remained normal. All animals were followed for 7 days. We have found no evidence that severe manipulation or temporary or permanent occlusion of the middle cerebral artery has produced delayed ischemic deficits in animal models. We therefore believe that this is not a factor in our primate model, particularly when it is considered that great care was taken to ensure minimal manipulation of the arteries involved.

Furthermore, animals in which a sham procedure was performed demonstrated no evidence of vasospasm at 7 days after operation.

Cerebral vasospasm with this model reaches a peak at 7 days post-subarachnoid hemorrhage (41). The animals in this trial were examined on Day 7 to evaluate the efficacy of nimodipine at the time of expected maximal vasoconstriction.

Espinosa et al. used nimodipine in a dose of 1 mg/kg p.o. q8h with this model and found no improvement in the incidence or severity of chronic vasospasm on the clot side when compared to placebo. There was a small dilatational effect observed on the non-clot side (41). The present study used dosages of 3, 6, and 12 mg/kg given orally every 8 hours. These doses are well above the reported clinically utilized dose (0.35 mg/kg p.o. q4h)(7). In spite of what may be considered massive dosages of nimodipine, there were no significant differences in the degree of vasospasm among groups, including placebo, on the clot side. Similarly, there was no difference observed between baseline and Day 7 cerebral vessel caliber among groups on the non-clot, control side, although there seemed to be a trend toward dilation. There was no significant difference among groups with respect to the development of collateral circulation.

Individual cases of mild vasospasm were occasionally noted on the non-clot side. This was expected due to the proximity of the clot to the midline. Some diffusion of vasospastic agents across the midline could occur. The hemorrhage, however, was induced with clotted blood, not liquid. This would tend to inhibit extensive diffusion of vasospastic agents throughout the subarachnoid space and

ensure that maximal vasospasm would occur in those vessels in direct contact with the clot.

One monkey receiving nimodipine, 12 mg/kg p.o. q8h, suffered a delayed ischemic deficit on Day 5. This animal showed no development of collateral circulation on the clot side, but did show mild dilation of vessels on the non-clot side.

A second animal, also from the nimodipine (12 mg/kg) group, suffered a reversible neurological deficit on Day 4 post-subarachnoid hemorrhage. The monkey developed a monoparesis of the left leg over 2 days. Function of the limb had markedly improved by late Day 7.

These data strongly suggest that the cerebral ischemia is delayed 4 to 5 days post-subarachnoid hemorrhage and is not the result of acute manipulations.

Both affected animals were from the highest dose nimodipine group. This group demonstrated a statistically significant drop in mean arterial blood pressure from Day 0 to Day 7. This may have been a factor in the development of the ischemia and infarction. However, other animals suffered greater decreases in mean arterial blood pressure, but sustained a lesser degree of vasospasm and did not suffer ischemia. Therefore, we think that the presence of vasospasm was the most significant factor in the development of the ischemia. In our previous studies with the same model (39, 42), the delayed ischemia and infarct that developed in one monkey did so in a placebo recipient.

The cerebral blood flow measurements showed no significant difference among groups or indeed between clot and non-clot cerebral hemispheres. This was probably the result of the large size and

parieto-occipital location of the scintillation detector equipment. Because only one detector was placed over each hemisphere, small changes in cerebral blood flow may not have been detected. Further, the development of collateral circulation in the clot side cerebral hemispheres probably negated any focal decrease in flow due to vasospasm. The next study (Aiv) used multiple, smaller area detectors in an attempt to detect regional variations in cerebral blood flow.

There was no difference among groups in the drop in hematocrit from Day 0 to Day 7. This decrease was therefore most likely a result of blood removal for clot formation and surgical losses. Diarrhea was a consistent finding in all groups, including placebo, and was therefore most likely due to the solvent, polyethylene glycol 400.

The morphological changes within the cerebral vessels of those animals studied with electron microscopy confirm the findings of Espinosa et al. (42). Some studies however, suggested that chronic cerebral vasospasm is not the result of sustained medial smooth muscle contractions, but rather results from histological changes such as subendothelial proliferation, intimal cellulofibrous thickening and organization of luminal thrombi (29, 30, 76, 116, 132, 171). The extent of the vascular morphological change is proportional to the amount of time after subarachnoid hemorrhage. Electron microscopy in studies of experimental subarachnoid hemorrhage have revealed vacuolization and fibrosis of the media, myonecrosis, thickened elastic laminae and rounding of endothelial cells (26, 44, 79, 92, 180, 184, 194).

Eldevik et al. (38) studied brain vessels obtained at autopsy and vessels from a dog model of vasospasm and could not confirm any morphological changes ascribed to vasospasm.

In a previous primate study, Espinosa et al. (42) found pathological changes such as endothelial ballooning and a "fish-scale" appearance of the endothelium. However, no mast cells were detected in the media, there was little inflammatory reaction, there were no intraluminal thrombi in spastic vessels, and there were no erythrocytes or debris evident in the media or intima. These authors concluded that vasospasm is the result of sustained contraction of the media smooth muscle cells in response to blood in the subarachnoid space. The present study of vessels in vasospasm at Day 7 post-subarachnoid hemorrhage confirms those findings. While the degree of spasm was greater in the present study (maximum vasospasm occurs on Day 7, Espinosa's study looked at the vessels on Day 14), there was no evidence of inflammatory response or intraluminal thrombi sufficient to cause angiographic narrowing of the vessels.

Furthermore, oral nimodipine at the dosages tested did not affect the incidence or severity of the changes observed.

The high performance liquid chromatography analysis of cerebrospinal fluid and serum nimodipine levels was inconclusive for determining definite dosage/level relationships. This was in part due to the large volumes of sample required for adequate analysis. This necessitated delaying the sample collection until just prior to sacrifice by which time the animals had received large volumes of intravenous fluids which were not necessarily consistent among the

animals. It was not possible to obtain sufficient volumes of cerebrospinal fluid to enable measurement of cerebrospinal fluid nimodipine levels. Nevertheless, the levels do suggest that nimodipine was present in the serum of all animals receiving oral nimodipine. It has been demonstrated that nimodipine given orally to humans is detectable in the cerebrospinal fluid within 2 hours of administration (13). Therefore it is probable that nimodipine was present in the cerebrospinal fluid of the animals in the nimodipine treatment groups. No nimodipine was detected in any of the placebo-group animals' sera or cerebrospinal fluid.

The cardiopulmonary hemodynamics of the animals in this study were also extensively evaluated. The results and conclusions are reported elsewhere (122).

The *in vitro* portion of this study was designed to resolve the question of whether arteries in vasospasm show enhanced reactivity as suggested by Svendgaard et al. (174) and Lobato et al. (101) or decreased reactivity as suggested by Toda et al. (186). It seems clear from our studies that, at least in the monkey, vessels in spasm show a reduced ability to contract to norepinephrine, 5-hydroxytryptamine, or potassium chloride. This presumably arises because there is a maximal force that can be exerted by the artery, and contractions to pharmacological agents are measured from a baseline that is elevated by the existence of vasospasm. In one animal that developed a stroke as a result of intense vasospasm, the arteries were almost devoid of any response and were the least reactive of all arteries tested. If some means of relaxing the arteries could be found, reactivity might be approximately normal or

even enhanced. The reported greater activity to biogenic amines of cat and rabbit arteries might arise from immediate relaxation of the arteries on removal from the animal, which would enable such enhanced activity to be observed (101, 174). However, in each model in which angiographic evidence of vasospasm is available, spastic arteries showed less activity. It is possible that, despite the correlation between development of supersensitivity and the expected time course of cerebral vasospasm, neither the cat nor the rabbit model actually produces this condition. It is also possible that species differences make the cat and rabbit unsuitable models of vasospasm in humans.

Two other issues arose from this aspect of the study. First, the middle cerebral artery from the monkey is much more responsive than that of the dog to norepinephrine in the control situation and yet its activity is depressed to the same extent by the other agents in the experimental situation. In both ED_{50} and the relative responsiveness with respect to other agonists, monkey artery seems to resemble human artery. This underscores the conclusions of Toda et al. that supersensitivity is an unlikely mechanism for cerebral vasospasm (40, 186). Furthermore, it seems that there is some lasting damage to the artery; even after prolonged washing, relaxation did not occur. This provides additional evidence against the idea that vasospasm arises from a normal artery either responding to the continued presence of an agonist in high concentration or becoming supersensitive to an agonist present in low concentration. It is important to note, however, that the decreased contractility of the spastic vessels may have been an artifact due to their

pre-contracted state at the time of *in vitro* testing. If the vessels could have been relaxed prior to evaluation of their contractile response, the results could conceivably have been different. Relaxation could possibly have been achieved by topical application of nimodipine in the organ bath (11).

The data with nimodipine are surprising in view of the partial success reported in the clinical trial by Allen et al. (7). Nimodipine seemed to reduce the incidence of severe neurological deficits in these patients, but all patients did not undergo repeat angiography. Therefore, the relationship to vasospasm reduction is still undetermined. In addition, the results reported in study A(*i*) of this work confirm the lack of success of even high doses of nimodipine in achieving a reduction in the degree of vasospasm when measured angiographically.

The effects of nimodipine became significant only when the non-clot side vessels were examined. Animals that had been treated with the highest dose of nimodipine tested (12 mg/kg p.o. q8h) showed an enhanced response to all agents tested. These effects were not due to the residual influence of the agent on calcium entry because this would have been expected to decrease the response. There are many data showing that calcium antagonists inhibit the response to the agents tested (21, 36, 160). The most probable hypothesis is that prolonged exposure to nimodipine, which would be expected to inhibit contraction of the artery, results in an adaptive response analogous to denervation supersensitivity. Further work is necessary to substantiate this suggestion.

These results are consistent with the first section of this study: treatment with nimodipine does not seem to affect the reactivity of arteries in spasm.

It is known that vessels from different anatomical sites within a given animal are not uniform in their response to various agonists. Not only is the cerebral vasculature pharmacologically different from the systemic, but it shows a wider interspecies variation than is observed in peripheral arteries. It was possible that although the vasospasm produced in the primate model was very similar to that seen in man, the monkeys' vessels could well demonstrate dissimilar responses to drugs.

Resistance of cynomolgus monkey vessels to dihydropyridine-type blockade would explain the lack of effect demonstrated in the first study. The third part of the study was therefore designed to characterize the monkey cerebral artery pharmacologic response and compare it to human and dog responses.

It has been established that nimodipine and other calcium blockers reduce the contractility of vascular smooth muscle to depolarizing agents in vessels from rabbit (178), cat (35), and dog (120) with an IC_{50} of 1.7×10^{-10}M to 5×10^{-9}M depending on the vessels tested. This study confirms that in the cerebral vessels of the monkey, dog, and man, nimodipine concentrations greater than 10^{-8}M abolished contractile response to potassium chloride, while concentrations of 10^{-9}M reduced the maximal response to about 50% of control values. While there is universal agreement that nimodipine is a potent antagonist of potassium-induced contractions, variable results have been reported about its effects on norepinephrine

responses. In rabbit basilar artery Takagi et al. (178) showed that nimodipine at concentrations up to 10^{-8}M had no effect on norepinephrine-induced contractions. Similar results were obtained by Kazda and Towart (87). On the other hand, this study demonstrated a high sensitivity to nimodipine in the norepinephrine-induced contractions of both dog and human arteries. Nimodipine 10^{-8}M reduced the response to less than 15% of the control value in each case, while the vessels from the monkey were much less affected. Muller-Schweinitzer and Newmann (120) reported that contractions of canine basilar arteries to 5-hydroxytryptamine were extremely sensitive to nimodipine. A concentration of 10^{-9}M nimodipine caused a 50% reduction in the maximum response. This corresponds exactly to the findings of the present study. This concentration of nimodipine did not affect the response of human or monkey arteries to 5-hydroxytryptamine, although a ten-fold increase in concentration causes dramatic inhibition of human vessels. The monkey is more resistant to the effects of nimodipine; 10^{-7}M produces only a 50% inhibition of the 5-hydroxytryptamine response in this species.

It thus seems that at least part of the failure of nimodipine to reverse cerebrovascular spasm in the *in vivo* model might arise from unexpected insensitivity of *Macaca fascicularis*. However, when the responses to hemoglobin and prostaglandin $F_{2\alpha}$ are examined, all species were resistant to the actions of nimodipine. Even at the high dose of 10^{-7}M, a substantial proportion of the response to prostaglandin $F_{2\alpha}$ remains. It is possible that hemoglobin may cause constriction by releasing prostaglandins and thus the insensitivity

of hemoglobin-induced contractions in all species may reflect the same phenomenon.

Brandt et al. (21) also reported similar findings in human pial arteries. The effects of prostaglandin $F_{2\alpha}$ were inhibited by nimodipine to a lesser extent than the effects of potassium. White et al. (210) examined canine basilar artery and reported that contractions elicited by blood, thrombin and hemoglobin were reduced by nimodipine (10^{-7}M) more than 50% although the antagonist had less effect on these agents than on 5-hydroxytryptamine. These authors did not construct full dose-response curves, and they tested nimodipine in a slightly different way, introducing it at the peak of the contraction or using it before a single large dose of the agonist. It is possible that different experimental circumstances can give slightly different results, but the tendency for prostaglandin-induced contractions to be resistant to nimodipine blockade, relative to contractions produced by other spasmogens, seems to be a general observation.

The reason for these observations presumably lies in the fact that potassium-induced contractions have an absolute requirement for an inward movement of calcium through nimodipine-sensitive, potential dependent channels while prostaglandin $F_{2\alpha}$ uses either a different and insensitive calcium channel or is able to activate intracellular stores of calcium directly. According to Brandt et al. (21), the latter is the correct explanation. A reason for the species difference in the case of norepinephrine and 5-hydroxytryptamine could be in the use of both mechanisms by these agonists. In the nimodipine-sensitive species the major effect would be on potential

dependent channels, while in resistant species, 5-hydroxytryptamine and norepinephrine might work by a similar mechanism to prostaglandin $F_{2\alpha}$.

It is important to note that the species variation observed can only account for the failure of nimodipine to reverse vasospasm in the *in vivo* model if the principal agent responsible for the production of cerebral vasospasm is norepinephrine, 5-hydroxytryptamine, or something similar. If, however, a cascade mechanism involving prostaglandins is responsible, nimodipine would not be expected to reverse vasospasm very well in any species.

The final part of the first series of the experiments was designed to confirm that nimodipine did cause an increase in cerebral blood flow in the cynomolgus monkey. Since the pharmacological studies had shown that the monkey cerebral arteries were less responsive to nimodipine blockade of some agonists, it was believed necessary to evaluate its cerebral blood flow response.

Increase in cerebral blood flow was recorded within 15 minutes of starting the 2 μg/kg/min and the 5 μg/kg/min nimodipine infusions. The percentage increase over basal cerebral blood flow rates agrees closely with those found by Harper et al. in the baboon (70). The simultaneous drop in mean arterial blood pressure could be hazardous in a clinical situation. However, though it was statistically significant, it was most likely not of such magnitude that appropriate hypertensive therapy would not counteract it.

If blood or its breakdown products are responsible for the induction of chronic cerebral vasospasm, then prompt, complete

evacuation of subarachnoid hematoma from the basal cisterns after subarachnoid hemorrhage should prevent its occurrence (127).

Clinical studies done by Japanese neurosurgeons have lent support to the premise that vigorous attempts at removal of subarachnoid hemorrhage clot decrease the incidence of post-subarachnoid hemorrhage vasospasm, as determined angiographically (179, 210), and delayed ischemic deficit (138). It is always difficult, however, to be sure that all subarachnoid blood has been evacuated during surgery since it is never known how much blood was lost to the subarachnoid space during the initial bleed. Furthermore, the liquid blood can flow to sites distant from the aneurysm before clotting and may be missed at operation. This study allowed direct visual placement of a known quantity of clotted blood into the subarachnoid space. This enabled a thorough, measured, evacuation of the clot at 24 hours. Surprisingly, although there was no doubt that a complete evacuation was performed under direct vision, the quantity of clot removed at 24 hours represented only approximately 32% of the weight of clot placed. This indicates that a tremendous absorption of water and possibly other components of the clot occurs within the first 24 hours after subarachnoid hemorrhage. The significance of this, if any, is unknown.

Attempts at lavage of the subarachnoid cisterns post-subarachnoid hemorrhage in an effort to remove clotted blood and decrease the incidence of vasospasm have had varying success (68, 78, 115, 123, 147, 148).

Recent studies by Alexander et al. (2) suggest that cisternal lavage 24 hours after subarachnoid hemorrhage in the 2-hemorrhage dog

model is not effective in preventing vasospasm. It was, however, effective in removing the majority of the gross clot. The authors suggest that any interaction of clot and vessel which results in vasospasm must occur prior to 24 hours post-subarachnoid hemorrhage. On the contrary, the present primate study shows that complete evacuation of clot at 24 hours post-subarachnoid hemorrhage is almost completely effective in preventing vasospasm. Whether model differences or earlier, more thorough clot removal accounts for this discrepancy is not known. Magnetic resonance imaging clearly demonstrated that total removal of the clot was achieved. In the monkey, no irreversible spasmogenic interaction of clot and vessel occurs within the first 24 hours post-subarachnoid hemorrhage.

It was anticipated that increasing the distribution of hemorrhage to the posterior circulation and to both sides of the circle of Willis would result in an increase in delayed ischemic deficits in this primate model. Furthermore, it was suspected that MRI might demonstrate any clinically silent infarcts which might have been missed in previous studies (41, and Study A of this thesis). While neither MRI nor histological examination revealed any silent infarcts due to vasospasm, they did detect a clinically unsuspected infarct likely due to surgical trauma. This demonstrates the sensitivity of MRI to ischemic pathology. The increase in the extent of the induced subarachnoid hemorrhage did result in a greater incidence of delayed ischemic deficit. The 25% of clot-group animals which suffered infarctions due to vasospasm more closely approximates the clinical experience than we have previously achieved with unilateral clot placement. The increase in yield probably results

from the involvement of the posterior circulation in the subarachnoid hemorrhage which thereby inhibits the development of collateral circulation. It was this collateral circulation which was believed to be the cause of the low yield of development of delayed ischemic deficit in previous studies using this model.

This study shows that prevention of vasospasm development is possible if complete removal of clot is performed within 24 hours of subarachnoid hemorrhage. It would, therefore, seem advisable to attempt as complete a removal of clot as is safely possible in subarachnoid hemorrhage patients operated upon within 24 hours of the initial ictus. However, the Northern American referral pattern does not allow the majority of subarachnoid hemorrhage patients to be operated upon within this time frame. Whether complete clot removal at times later than 24 hours is effective in preventing vasospasm, or whether a definitive critical time exists after which clot removal does not alter the course of vasospasm, is as yet unknown. If complete clot removal later than 24 hours proves not to be effective, it may still augment the action of other vasolytic therapies such as cisternal lavage and parenteral or topical calcium blockers.

It is unfortunate that the inherent variability in the cerebral vessels themselves and in the radioimmunoassay technique could not more clearly demonstrate differences among all three groups in the animals tested. It was possible to demonstrate that subarachnoid hemorrhage induction and vasospasm development has no effect on basal thromboxane B_2 levels. It was also possible to demonstrate a clear statistical difference between the clot and clot-removal groups' arteries' basal levels of 6-keto-prostaglandin $F_{1\alpha}$. The

disappointing factor was that the sham group 6-keto-prostaglandin $F_{1\alpha}$ levels lay between the clot and clot-removal groups'. While we can speculate that larger sample sizes would have merged the results of the sham group with one of the other two, it is not possible to predict which way the results would develop. The data do not allow determination of whether the clot group 6-keto-prostaglandin $F_{1\alpha}$ levels represent a decrease in production from normal or whether the clot-removal group's production represents an increase from normal. While the first hypothesis is the most attractive teleologically, it is arguable that the clot-removal group may have been stimulated into increased production.

Further work will be required to delineate this problem; however, it can be safely stated that 6-keto-prostaglandin $F_{1\alpha}$ (and therefore, prostacyclin) synthesis is affected by subarachnoid hemorrhage and vasospasm.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

The conclusions from these studies can be divided into two groups based on the two series of this investigation. The first series demonstrated that oral nimodipine at the doses tested was not effective in reducing the incidence or severity of chronic cerebral vasospasm in the monkey model. It was also not effective in preventing a decrease in reactivity to several agonists of vessels in spasm. Chronic administration did, however, enhance the *in vitro* reactivity of non-spastic vessels. The cerebral vessels from *Macaca fascicularis* are less sensitive to blockade by nimodipine to activity caused by norepinephrine and 5-hydroxytryptamine. None of human, dog, or monkey vessels are sensitive to nimodipine blockade against contractions induced by prostaglandin $F_{2\alpha}$ or hemoglobin, the most likely mediators of vasospasm.

Intravenous nimodipine does cause dose-related increase in cerebral blood flow in the monkeys tested and results in a decrease in systemic blood pressure.

It is increasingly likely from human studies that nimodipine does exert a beneficial effect in the overall course of the sequelae to subarachnoid hemorrhage. If this is so, improvement may arise from an alteration in cardiovascular status, a protective effect on neurons, or by the dilation of a non-spastic collateral circulation.

The second series of experiments demonstrated that complete removal of subarachnoid blood within 24 hours of a subarachnoid hemorrhage markedly decreases the incidence of vasospasm.

The development of the primate model to include extension of the induced hemorrhage to the posterior circulation increased the incidence of delayed ischemic events to 25%.

Analysis of the basal production levels of thromboxane A_2 and prostacyclin of normal cerebral vessels and those in spasm revealed there was no difference in thromboxane A_2 levels but that in vessels from which the clot had been removed, prostacyclin levels were significantly greater than those of vessels in spasm.

These studies indicate that the optimal method for preventing vasospasm after subarachnoid hemorrhage is to operate within 24 hours and to attempt removal of as much clot as possible. The thorough removal that was accomplished in the experimental model is seldom possible in the clinical situation. Therefore, continued research into means of treating vasospasm as it develops (or before it develops) is required. It is possible that removal of as much clot as is safely possible may decrease the spasmogenic load on the cerebral vessels such that alternate therapies such as calcium blockade, topical vasodilators, or cisternal lavage may be of greater use.

It is also still necessary to determine whether there is a finite time, later than 24 hours, after which clot evacuation will not prevent development of vasospasm.

The discovery of an effective, simple, low morbidity method of preventing or treating chronic cerebral vasospasm would be a boon to the management of subarachnoid hemorrhage due to aneurysm rupture. The frustration and disappointment of watching a neurologically intact subarachnoid hemorrhage patient deteriorate or die because of

the development of vasospasm cannot be overstated. The cost to the patient is obvious. What is not so obvious is the cost to society of losing up to 10,000 productive individuals per year in North America alone.

Although a panacea is yet to be discovered, the current thrust of research promises to uncover new therapeutic strategies. The primate model of chronic cerebral vasospasm after subarachnoid hemorrhage will facilitate the rapid evaluation of present and proposed future vasolytic therapies. This will provide effective therapies a rapid assimilation to the clinician's armamentarium.

BIBLIOGRAPHY

1 Allcock JM, Drake CG: Ruptured intercranial aneurysms--the role of arterial spasm. *J Neurosurg* 22:21-29, 1965.

2 Alexander III E, Black PM, Liszczak TM, Zervas NT: Delayed CSF lavage for arteriographic and morphological vasospasm after experimental SAH. *J Neurosurg* 63:949-958, 1985.

3 Alksne JF, Branson PJ: Prevention of experimental subarachnoid hemorrhage-induced intracranial arterial vasonecrosis with phosphodiesterase inhibiter phthalazinol (EG-626). *Stroke* 10:638-644, 1979.

4 Allen GS: Cerebral arterial spasm: A discussion of present and future research. *Neurosurgery* 1:142-148, 1977.

5 Allen GS: Cerebral arterial spasm: Update on treatment of delayed ischemic neurological deficits with sodium nitroprusside and phenylephrine, in: Wilkins RH (ed): *Cerebral Arterial Spasm.* Baltimore: Williams & Wilkins, 1980, pp. 589-590.

6 Allen GS: Cerebral Arterial Spasm, Part 8: The treatment of delayed cerebral arterial spasm in human beings. *Surg Neurol* 6:71-80, 1976.

7 Allen GS, Ahn MS, Preziosi TJ, Battye R, Boone SC, Chou SN, Kelly DL, Weir BK, Crabbe RA, Lavik PJ, Rosenbloom SB, Dorsey FC, Ingram CR, Mellits DE, Bertsch LA, Boisvert D, Hundley MB, Johnson RK, Strom JOA, Transou CR: Cerebral arterial spasm: A controlled trial of nimodipine in subarachnoid hemorrhage patients. *N Engl J Med* 308: 619-624, 1983.

8 Allen GS, Gross CJ, Henderson LM, Chou SN: Cerebral arterial spasm: Part 4. *In vitro* effects of temperature, serotonin analogues, large non-physiological concentrations of serotonin and extracellular calcium and magnesium on serotonin-induced contractions of the canine basilar artery. *J Neurosurg* 44:585-593, 1976.

9 Asano T, Saskai T, Ochiai C, Takakura K: *In vitro* evaluation of the inhibitory action of PGI_2 to vasoconstriction induced by various prostaglandins, serotonin and hemoglobin using the canine basilar artery. *Neurol Med Chir (Tokyo)* 22:507-512, 1982.

10 Asano T, Tanishima T, Saskai T, Sano K: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage, in: Wilkins RH (ed): *Cerebral Arterial Spasm.* Baltimore: Williams & Wilkins, 1980, pp. 190-201.

11 Auer LM, Ito Z, Suzuki A, Ohta H: Prevention of symptomatic vasospasm by topically applied nimodipine. *Acta Neurochir (Wein)* 63:297-302, 1982.

12 Baena RR, Gaetani P, Folco G, Branzoli U, Paoletti P: Cisternal and lumbar CSF concentration of arachidonate metabolites in vasospasm following subarachnoid hemorrhage from ruptured aneurysm: Biochemical & clinical considerations. *Surg Neurol* 24:428-432, 1985.

13 Battye R: Plasm and cerebrospinal fluid levels after oral doses of nimodipine, in: Battye R (ed): *Calcium Antagonists: Possible Therepeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp. 62-64.

14 Bevan JA: Calcium & vascular tone, in: Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp. 6-14.

15 Blumenkopf B, Wilkins RH, Feldman JM: Cerebral vasospasm and delayed neurological deficit after aneurysm rupture despite administration of reserpine and kanamycin, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 518-524.

16 Blumenkopf B, Wilkins RH, Feldman JM: Cerebral vasospasm despite administration of reserpine and kanamycin. *Neurosurgery* 4:548, 1979.

17 Boisvert DPJ: Regional cerebral blood flow autoregulation and cerebral vasospasm in monkeys with subarachnoid hemorrhage. PhD Thesis, University of Alberta, 1978.

18 Boullin DJ: Vasospasm and prostacyclin - a new hypothesis and possibilities for therapy, in Boullin DJ (ed): *Cerebral Vasospasm*. New York: John Wiley, 1980, pp. 209-229.

19 Boullin DJ, Brandt L, Ljunggren B, Tagari P: Vasoconstrictor activity in cerebrospinal fluid from patients subjected to early surgery for ruptured intracranial aneurysms. *J Neurosurg* 55:237-245, 1981.

20 Boullin DJ, Bunting S, Blaso WP, Hunt TM, Moncada S: Responses of human and baboon arteries to prostaglandin endoperoxides and biologically generated and synthetic prostacyclin: Their relevance to cerebral arterial spasm in man. *Brit J Clin Pharmacol* 7:139-147, 1979.

21 Brandt L, Andersson K-E, Edvinsson L, Ljunggren B: Effects of extracellular calcium and of calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. *J Cereb Blood Flow Metab* 1:339-347, 1981.

22 Brandt L, Ljunggren B, Andersson K-E, Hindfelt B, Uski T: Effects of indomethacin and prostacyclin on isolated pial arteries contracted by CSF from patients with aneurysmal SAH. *J Neurosurg* 55:877-833, 1981.

23 Brandt L, Ljunggren B, Andersson K-E, Hindfelt B, Uski T: Prostaglandin metabolism and prostacyclin in cerebral vasospasm. *Gen Pharmacol* 14:141-143, 1983.

24 Camp PE, Paxton HD, Buchan GC, Gahbauer H: Vasospasm after trans-sphenoidal hypophysectomy. Case Report. *Neurosurgery* 7:382-386, 1980.

25 Chapleau CE, White RP, Robertson JT: Cerebral vasospasm: effects of prostaglandin synthetase inhibitors *in vitro*. *Neurosurgery* 6:155-159, 1980.

26 Clower BR, Haining JL, Smith RR: Pathophysiological changes in the cerebral artery after subarachnoid hemorrhage, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 124-131.

27 Cohen RJ, Allen GS: Cerebral arterial spasm: the role of calcium in *in vitro* and *in vivo* analysis of treatment with nifedipine & nimodipine, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 527-532.

28 Cook DA, Bielkiewicz B: A computer-assisted technique for analysis & comparison of dose response curves. *J Pharmacological Methods* 11:77-89, 1984.

29 Conway LW, McDonald LW: Structural changes of the intradural arteries following subarachnoid hemorrhage. *J Neurosurg* 37:715-723, 1972.

30 Crompton MR: The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. *Brain* 87:491-510, 1964.

31 DaPian R, Pasqualin A, Scienza R, Licata C, Cavazzani P: A preliminary report on early surgery for bleeding intracranial aneurysms. *J Neurosurgery Sci* 27:1-8, 1983.

32 Drake CG: Formal discussion on Symon L: Vasospasm in aneurysm, in: Moosy J, Janeway R (eds): *Cerebrovascular Diseases, Seventh Conference*. New York: Grune and Stratton. pp. 241-244.

33 Drake CG: Management of cerebral aneurysm. *Stroke* 12:273-283, 1981.

34 Ecker A, Riemenschneider PA: Arteriographic demonstration of spasm of the intracranial arteries with special reference to saccular arterial aneurysms. *J Neurosurg* 8:660-667, 1951

35 Edvinsson L, Andersson K-E, Brandt L, Ljunggren B, MacKenzie ET, Skarby T: Effects of Ca^{++} antagonists on cerebral blood vessels. *J Cereb Blood Flow Metab* 1(Suppl 1): S344-345, 1981.

36 Edvinsson L, Brandt L, Andersson K-E, Bengtsson B: Effect of a calcium antagonist on experimental constriction of human brain vessels. *Surg Neurol* 11:327-330, 1979.

37 Ellis EF, Nies AS, Oates JA: Cerebral arterial smooth muscle contractions by thromboxane A_2. *Stroke* 8:480-483, 1977.

38 Eldevik OP, Kristiansen K, Torvik A: Subarachnoid hemorrhage and cerebrovascular spasm. Morphological study of intracranial arterials based on animal experiments and human autopsies. *J Neurosurg* 55:869-876, 1981.

39 Espinosa F: A primate model of chronic cerebral vasospasm: Development and application in the treatment of subarachnoid hemorrhage in humans. PhD Thesis, University of Alberta, 1985, p. 63.

40 Espinosa F, Weir B, Boisvert D, Overton T, Castor W: Chronic cerebral vasospasm after large subarachnoid hemorrhage in monkeys. *J Neurosurg* 57:224-232, 1982.

41 Espinosa F, Weir B, Overton T, Castor W, Grace M, Boisvert D: A randomized, placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part I: Clinical and radiological findings. *J Neurosurg* 60:1167-1175, 1984.

42 Espinosa F, Weir B, Shnitka T, Overton T, Boisvert D: A randomized placebo-controlled trial of nimodipine after subarachnoid hemorrhage in monkeys: Part II: Pathological findings. *J Neurosurg* 60:1176-1185, 1984.

43 Farhat SM, Schneider RC: Observations on the effect of systemic blood pressure on intracranial circulation in patients with cerebrovascular insufficiency. *J Neurosurg* 27:441-445, 1967.

44 Fein JM, Flor WJ, Cohan SL, Parkhurst J: Sequential changes of vascular ultrastructure in experimental cerebral vasospasm. Myonecrosis of subarachnoid arteries. *J Neurosurg* 41:49-58, 1974.

45 Fisher CM: Clinical syndromes in cerebral thrombosis, hypertensive hemorrhage, and ruptured saccular aneurysm. *Clin Neurosurg* 22:117-147, 1975.

46 Fisher CM, Roberson GH, Ojemann RG: Cerebral vasospasm with ruptured saccular aneurysm - the clinical manifestations. *Neurosurgery* 1:245-248, 1977.

47 Flaim ES, Kim J, Lin J, Ransohoff: Phosphodiesterase inhibitors and cerebral vasospasm. *Arch Neurol* 32:569-571, 1975.

48 Flaim SF: Comparative pharmacology of calcium blockers based on studies of vascular smooth muscle, in: Flaim SF, Zelis R (eds): *Calcium Channel Blockers: Mechanisms of Action and Clinical Applications*. Baltimore: Urban & Schwarzenberg, 1982, pp. 155-178.

49 Fleckenstein A: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Annu Rev Pharmacol Toxicol* 17:149-166, 1977.

50 Fleckenstein A, Nakayama K, Fleckstein-Grun G, Byon YK: Interactions of H ions, Ca-antagonistic drugs and cardiac glycosides with excitation-contraction coupling of vascular smooth muscle, in: Betz E (ed): *Ionic Actions on Vascular Smooth Muscle*. New York: Springer-Verlag, 1976, pp. 117-123.

51 Fleckenstein A, Nakayama K, Fleckenstein-Grun G, Byon YK: Interactions of vasoactive ions and drugs with Ca-dependent excitation-contraction coupling of vascular smooth muscle, in: Carafoli E (ed): *Calcium Transport in Contractions and Secretions*. Amsterdam: North Holland, 1975, pp. 555-566.

52 Forster C, Whalley ET: Possible functional role of fibrinogen degradation products in cerebral arterial spasm. *J Cereb Blood Flow Metab* 1(Suppl 1):S564, 1981.

53 Forster C, Whalley ET, Mohan J, Dutton J: Vascular smooth muscle response to fibrinogen degradation products and 5-hydroxytryptamine: Possible role in cerebral vasospasm in man. *Br J Clin Pharmacol* 10:231-236, 1980.

54 Fujita K, Shingu T, Gi H, Araki O, Mutsunaga M, Handa H: Mechanism of cerebral vasospasm induced by oxyhemoglobin, in: Brock M (ed): *Modern Neurosurgery*. Berlin: Springer-Verlag, 1982, pp. 359-374.

55 Fukumori T, Tani E, Maeda Y, Sukenaga A: Effects of prostacyclin and indomethacin on experimental delayed cerebral vasospasm. *J Neurosurg* 59:829-834, 1983.

56 Fukumori T, Tani, Maeda Y, Sukenaga A: Effect of selective inhibitor of thromboxane A_2 synthetase on experimental cerebral vasospasm. *Stroke* 15:306-311, 1984.

57 Furchgott RF: Role of the endothelium in responses of vascular smooth muscle. *Circulation Research* 53:557-573, 1983.

58 Gaab MR, Barwanski A, Backhorn J, Haubitz I, Rode ChP, Maximillian VA: Calcium antagonism: A new therapeutic principle in stroke and cerebral vasospasm. *rCBF Bull* 3:47-51, 1982.

59 Gaudet RJ, Levin L: Transient cerebral ischemia and brain prostaglandins. *Biochem Biophys Res Commun* 86:893-901, 1979.

60 Gavras H, Andrews P, Papadakis N: Reversal of experimental--delayed cerebral vasospasm by angiotensin-converting enzyme inhibition. *J Neurosurg* 55:884-888, 1981.

61 Gelmers HJ: Effect of nimodipine (Bay e 9736) on postischemic cerebrovascular reactivity, as revealed by measuring regional cerebral blood flow (rCBF). *Acta Neurochir (Wein)* 63:283-290, 1982.

62 Giannotta SL, McGillicuddy JE, Kindt GW: Diagnosis and treatment of postoperative cerebral vasospasm. *Surg Neurol* 8:286-290, 1977.

63 Graf CJ, Nibbelink DW: Cooperative study of intracranial aneurysms and subarachnoid hemorrhage. Report on a randomized treatment study. III. Intracranial Surgery. *Stroke* 5:559-601, 1974.

64 Graham DI, MacPherson P, Pitts LH: Correlation between angiographic vasospasm, hematoma, and ischemic brain damage following SAH. *J Neurosurg* 59:223-230, 1983.

65 Grotenhuis JA, Bettag W, Feibach BJO, Dabir K: Intracarotid slow bolus injections of nimodipine during angiography for treatment of cerebral vasospasm after SAH. A preliminary report. *J Neurosurg* 61:231-240, 1984.

66 Grubb, RL Jr: Cerebral hemodynamics and metabolism in subarachnoid hemorrhage and vasospasm, in: Wilkins RH (ed): *Cerebral Arterial Vasospasm*. Baltimore: Williams & Wilkins, 1980, pp. 341-349.

67 Hagan AA, White RP, Robertson JT: Synthesis of prostaglandin and thromboxane B_2 by cerebral arteries. *Stroke* 10:306-309, 1979.

68 Hamer J: The significance of cerebral vasospasm with regard to early and delayed aneurysmal surgery. Preliminary results of early surgery. *Acta Neurochir (Wein)* 63:209-213, 1982.

69 Handa H, Osaka K, Okamoto S: Breakdown products of erythrocytes as a cause of cerebral vasospasm, in: Wilkins RM (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 158-165.

70 Harper AM, Craigen L, Kazda S: Effect of the calcium antagonist, nimodipine, on cerebral blood flow and metabolism in the primate. *J Cereb Blood Flow Metab* 1:349-356, 1981.

71 Harris RJ, Branston NM, Symon L, Bayhan M, Watson A: The effects of a calcium antagonist, nimoidipine, upon physiological responses of the cerebral vasculature and its possible influence upon focal cerebral ischemia. *Stroke* 13:759-766, 1982.

72 Hashi K, Matsuoka Y, Tanaka K, Ohkawa N: Treatment of cerebral vasospasm with large doses of hydrocortisone, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 611-618.

73 Heros RC, Zervas NT, Negoro M: Cerebral vasospasm. *Surg Neurol* 5:354-362, 1976.

74 Heros RC, Zervas NT, Varsos V: Cerebral vasospasm after subarachnoid hemorrhage: an update. *Ann Neurol* 14:599-608, 1983.

75 Horton EW: Prostaglandins and smooth muscle. *Br Med Bull* 35:295-300, 1979.

76 Hughes JT, Schianchi PM: Cerebral Arterial Spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. *J Neurosurg* 48:515-525, 1978.

77 Jarman DA, DuBoulay GH, Kendall B, Boullin DJ: Responses of baboon cerebral and extracèrebral arteries to prostacyclin and prostaglandin endoperoxide *in vitro* and *in vivo*. *J Neurol Neurosurg Psychiatry* 42:677-687, 1979.

78 Johnson R, Potter JF, Reid RG: Arterial spasm and subarachnoid hemorrhage: Mechanical considerations. *J Neurol Neurosurg Psychiatry* 21:68, 1958.

79 Joris I, Majno G: Endothelial changes induced by arterial spasm. *Am J Pathol* 102:346-358, 1981.

80 Kamiyama K, Okada H, Suzuki J: The relation between cerebral vasospasms and superoxide - Application of an experimental model of vasospasms induced by oxyhemoglobin in cats. *Neurol Med Chir (Tokyo)* 21:201-209, 1981.

81 Kapp JP, Mahaley Jr. MS, Odom GL: Cerebral Arterial Spasm. Part 2: Experimental evaluation of mechanical and humoral factors in pathogenesis. *J Neurosurg* 29:339-349, 1968.

82 Kassell NF: The natural history and treatment outcome of SAH: comments derived from the national cooperative aneurysm study, in: Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp. 24-29.

83 Kassell N, Boarini DJ, Adams HP, Sahs AL, Graf CJ, Torner JC, Gerk MK: Overall management of ruptured aneurysm: Comparison of early and late operation. *Neurosurgery* 9:120-128, 1981.

84 Kassell NF, Peerless SJ, Durward QJ, Beck DW, Drake CG, Adams HP: Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. *Neurosurgery* 11:337-343, 1982.

85 Kassell NF, Torner JC: Epidemiology of intracranial aneurysms. *Int Anaes Clin* 20:13-17, 1982.

86 Kazda S, Hoffmeister F, Garthoff B, Towart R: Prevention of the postischemic impaired reperfusion of the brain by nimodipine (Bay e 9736). *Acta Neurol Scand* 60(Suppl 72):302-303, 1979.

87 Kazda S, Towart R: Nimodipine: a new calcium antagonistic drug with a preferential cerebrovascular action. *Acta Neurochir (Wein)* 63:259-265, 1982.

88 Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zervas N, Fisher CM: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan. A prospective study. *Neurology* 33:424-436, 1983.

89 Kukovetz WR, Poch G, Holzmann S: Cyclic nucleotides and relaxation of vascular smooth muscle, in: Vanheutte PM, Leusen I (ed): *Vasodilitation*. New York: Raven Press, 1981, pp. 339-353.

90 La Torre E, Patrono C, Fortuna A, Grossi-Belloni: Role of prostaglandin F_2 in human cerebral vasospasm. *J Neurosurg* 41:293-299, 1974.

91 Laurent JP: Subarachnoid hemorrhage, in: Wood JH (ed): *Neurobiology of CSF*. New York: Plenum Press, 1980, pp. 279-285.

92 Liszczak TM, Varsos VG, Black PM, Kistler JP, Zervas NT: Cerebral Arterial Constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. *J Neurosurg* 58:18-26, 1983.

93 Little JR: Modification of acute focal ischaemia by treatment with mannitol and high dose dexamethasone. *J Neurosurg* 49:517-524, 1978.

94 Little JR, Sundt TM, Kerr FWL: Neuronal alterations in developing cortical infarction. An experimental study in monkeys. *J Neurosurg* 40:186-198, 1974.

95 Ljunggren B, Brandt L, Saveland H, Nilsson PE, Cronqvist S, Andersson K-E, Vinge E: Outcome in 60 consecutive patients treated with early aneurysm operation and intravenous nimodipine. *J Neurosurg* 61:864-873, 1984.

96 Ljunggren B, Brandt L, Sundbarg G, Saveland H, Cronqvist S, Stridbeck H: Early management of aneurysmal subarachnoid hemorrhage. *Neurosurgery* 11:412-418, 1982.

97 Ljunggren B, Saveland H, Brandt L: Causes of unfavorable outcome after early aneurysm operation. *Neurosurgery* 13:629-633, 1983.

98 Loach AB, Benedict CR: Plasmic catacholamine concentrations associated with cerebral vasospasm. *J Neurol Sci* 45:261-271, 1980.

99 Lobato RD, Marin J, Salaices M, Rico ML, Sanchez CF: Effect of subarachnoid hemorrhage on contractile responses and noradrenaline release evoked in cat cerebral arteries by histamine. *J Neurosurg* 55:543-549, 1981.

100 Lobato RD, Marin J, Salaices M, Burgos J, Rivilla F, Garcia AG: Effect of experimental subarachnoid hemorrhage on the adrenrenergic innervation of cerebral arteries. *J Neurosurg* 53:477-479, 1980.

101 Lobato RD, Marin J, Salaices M, Rivilla F, Burgos J: Cerebrovascular reactivity to noradrenaline and serotonin following experimental subarachnoid hemorrhage. *J Neurosurg* 53:480-485, 1980.

102 Locksley HB: Report on the cooperative study of intracranial aneurysms and subarachnoid hemorrhage. Section V, Part II. Natural history of subarachnoid hemorrhage, intracranial aneurysms and arteriovenous malformations. Based on 6368 cases in the Cooperative Study. *J Neurosurg* 25:321-368, 1966.

103 Lye RH, Paul KS, Forster CM, Whalley ET, Dutton J: Effect of fibrin-fibrinogen degradation products on human basilar artery preparations. Possible role in the etiology of cerebral arterial spasm. *J Neurosurg* 56:339-342, 1982.

104 Lyons EL, Leeds NE: The angiographic demonstration of arterial vascular disease in purulent meningitis. *Radiology* 88:935-938, 1967.

105 Maeda Y, Tani E, Miyamoto T: Prostaglandin metabolism in experimental cerebral vasospasm . *J Neurosurg* 55:779-785, 1981.

106 Marcus AJ, Broekman MJ, Weksler BB, Jaffe EA, Safier LB, Ullman HL, Islam N, Tack-Goldman K: Arachidonic acid metabolism in endothelial cells and platelets. *Ann NY Acad Sci* 401:195-202, 1982.

107 Marshall LF, Bruce DA, Bruno L, Langfitt TW: Vertebrobasilar spasm. A significant cause of neurological deficit in head injury. *J Neurosurg* 48:560-564, 1978.

108 Maspes PE, Marini G: Intracranial arterial spasm related to supraclinoid ruptured aneurysms. *Acta Neurochir (Wein)* 10:630-638, 1962.

109 Matthews WF, Frommeyer Jr WB: The *in vitro* behaviour of erythrocytes in human cerebrospinal fluid. *J Lab Clin Med* 45:508-515, 1955.

110 Mawk JR, Ausman JI, Erickson DL, Maxwell RE: Vasospasm following transcranial removal of large pituitary adenomas. Report of three cases. *J Neurosurg* 50:229-232, 1979.

111 McCalden TA, Bevan JA: Sources of activator calcium in the rabbit basilar artery. *Am J Physiol* 241:H129-H133, 1981.

112 McIlhany MP, Johns LM, Leipzig T, Patronas NJ, Brown FD, Mullan SF: *In vivo* characterization of vasocontractile activities in erythrocytes. *J Neurosurg* 58:356-361, 1983.

113 Mickey B, Vorstrup S, Voldby B, Lindewald H, Harmsen A, Lassen NA: Serial measurement of regional cerebral blood flow in patients with SAH using ^{133}Xe inhalation and emission computerized tomography. *J Neurosurg* 60:916-922, 1984.

114 Millikan CH: Cerebral vasospasm and ruptured intracranial aneurysm. *Arch Neurol* 32:433-449, 1975.

115 Mizukami M, Kawase T, Usami T, Tazawa T: Prevention of vasospasm by early operation with removal of subarachnoid blood. *Neurosurgery* 10:301-307, 1982.

116 Mizukami M, Kin H, Araki G, Mihara H, Yoshida Y: Is angiographic spasm real spasm? *Acta Neurochir (Wein)* 34:247-259, 1976.

117 Mizukami M, Usami T, Tazawa T, Kawase T: Prevention of vasospasm by removal of subarachnoid blood in early operation. Neurol Med Chir (Tokyo) 21:1069-1077, 1981.

118 Moncada S, Bunting S, Mullane K, Thorogood P, Vane JR, Raz A, Needlemen P: Imidazole: A Selective Inhibitor of Thromboxane synthetase. *Prostaglandins* 13:611-618, 1977.

119 Morooka H: Cerebral artérial spasm II. Etiology and treatment of experimental cerebral vasospasm. *Acta Med Okayama* 32:39-49, 1978.

120 Muller-Schweinitzer E, Neumann P: 'In vitro' effects of calcium antagonists PN 200-110, nifedipine, and nimodipine on human and canine cerebral arteries. *J Cereb Blood Flow Metab* 3:354-361, 1983.

121 Nakayama K, Kato H: Different calcium dependancy of contractile responses of cerebral artery to quick stretch and electric stimuli. *Acta Neurol Scand* 60(suppl 72):508-509, 1979.

122 Norris S: Cardiopulmonary effects of nimodipine after subarachnoid hemorrhage in monkeys. MSc Thesis, University of Alberta, 1984.

123 Ohta H, Ito Z, Yasui N, Suzuki A: Extensive evacuation of subarachnoid clot for prevention of vasospasm - effective or not? *Acta Neurochir (Wein)* 63:111-116, 1982.

124 Okamoto S, Handa H, Toda N: Role of Intrinsic arachidonate metabolites in the vascular action of erythrocyte breakdown products. *Stroke* 15:60-64, 1984.

125 Okwuasaba FK, Cook D, Weir B: Changes in vasoctive properties of blood products with time and attempted identification of the spasmogens. *Stroke* 12:775-780, 1981.

126 Okwuasaba FK, Weir BK, Cook DA, Krueger CA: Effects of various intracranial fluids on smooth muscle. *Neurosurgery* 9:402-406, 1981.

127 Osaka K: Prolonged vasospasm produced by the breakdown products of erythrocytes. *J Neurosurg* 47:403-411, 1977.

128 Ott E, Lechner H: The influence of nimodipine on cerebral blood flow in patients with subacute cerebral infarction, in: Betz E, Grote D, Heuser D, Wallenweber R, (eds): *Pathophysiology and Pharmacotherapy of Cerebrovascular Disorders. Satellite Symposium of the XXVIII International Congress of Physiological Sciences*, 1980.

129 Pakarinen S: Incidence, aetiology, and prognosis of primary subarachnoid hemorrhage. A study based on 589 cases diagnosed in a defined urban population during a defined period. *Acta Neurol Scand* 43(Suppl 29):1-28, 1967.

130 Paul KS, Whalley ET, Forster C, Lye R, Dutton J: Prostacyclin & cerebral vessel relaxation. *J Neurosurg* 57:334-340, 1982.

131 Pearce WJ, Bevan JA: The cerebrovascular selectivity of diltiazem. *Program Ann Meeting, Am Assoc Neurol Surg.*, Washington, DC, 1983, pp. 60-61.

132 Peerless SJ, Kassell NF, Komatsu K, Hunter IG: Cerebral vasospasm: acute proliferative vasculopathy? II. Morphology, in: Wilkins RH (ed): *Cerebral Arterial Spasm.* Baltimore: Williams & Wilkins, 1980, pp. 88-96.

133 Pena H, Gaines C, Suess D, Crowell RM, Waggener JD, DeGirolami U: Effect of mannitol on experimental focal ischemia in awake monkeys. *Neurosurgery* 11:477-481, 1982.

134 Peterson EW, Searle R, Mandy FF, Leblanc R: The reversal of experimental vasospasm by dibutyryl-3', 5'-adenosine monophosphate. *J Neurosurg* 39:730-734, 1973.

135 Petruk KC: Cerebral circulatory responses to subarachnoid hemorrhage in the rhesus monkey. PhD Thesis, University of Alberta, 1976.

136 Phillips II LH, Whisnant JP, O'Fallon WM, Sundt TM: The unchanging pattern of subarachnoid hemorrhage in a community. *Neurology* 30:1034-1040, 1980.

137 Pitts LH, MacPherson P, Wyper DJ, Jennett B, Blair I, Cooke MBD: Effects of vasospasm on cerebral blood flow after subarachnoid hemorrhage, in: Wilkins RH (ed): *Cerebral Arterial Spasm.* Baltimore: Williams & Wilkins, 1980, pp. 333-337.

138 Pool JL: Early treatment of ruptured intracranial aneurysms of the circle of Willis with special clip technique. *Bull NY Acad Med* 35:357-369, 1959.

139 Quintana L, Konda R, Ishibashi Y, Yoshimoto T, Suzuki J: The effect of prostacyclin on cerebral vasospasm. An experimental study. *Acta Neurochir (Wein)* 62:187-193, 1982.

140 Ritchie WL: The pathophysiology and treatment of subarachnoid hemorrhage in the cynomolgous monkey. MSc Thesis, University of Alberta, 1979.

141 Ropper AH, Zervas NT: Outcome 1 year after SAH from cerebral aneurysm. Management morbidity, mortality, and functional status in 112 consecutive good-risk patients. *J Neurosurg* 60:909-915, 1984.

142 Rothberg CS: Pathophysiology and treatment of subarachnoid hemorrhage in the cynomolgous monkey. PhD Thesis, University of Alberta, 1979.

143 Rousseax P, Scherpereel B, Bernard MH, Graftieaux JP, Guyot JF: Fever and cerebral vasospasm in ruptured intracranial aneurysms. *Surg Neurol* 14:459-465, 1980.

144 Saito I, Sano K: Vasospasm after aneurysm rupture: Incidence, onset and course, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 294-301.

145 Sandahl B, Andersson K-E, Aronsen KF, Ulmsten U: Effect of the calcium antagonist nifedipine on uterine blood flow in non-treated and oestradiol-treated rats. *Gynecol Obstet Invest* 9:238-243, 1978.

146 Sano K: Timing of surgery: The Japanese experience, in: Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communication, 1983, pp. 35-42.

147 Sano K, Saito I: Early operation and washout of blood clots for prevention of cerebral vasospasm, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 510-513.

148 Sano K, Saito I: Timing and indication of surgery for ruptured intracranial aneurysms with regard to cerebral vasospasm. *Acta Neurochir (Wein)* 41:49-60, 1978.

149 Sasaki T, Asano T, Sano K, Murota S: Evaluation of prostacyclin biosynthetic activity in canine basilar artery following subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 1:560-561, 1981.

150 Sasaki T, Asano T, Takakura K, Sano K, Kassell NF: Nature of vasoactive substance in CSF from patients with subarachnoid hemorrhage. *J Neurosurg* 60:1186-1191, 1984.

151 Sasaki T, Mayanagi Y, Yano H, Kim S: Cerebral vasospasm with subarachnoid hemorrhage from cerebral arterioveneous malformation. *Surg Neurol* 16:183-187, 1981.

152 Sasaki T, Murota S-I, Wakai S, Asano T, Sano K: Evaluation of prostaglandin biosynthetic activity in canine basilar artery following subarachnoid injection of blood. *J Neurosurg* 55:771-778, 1981.

153 Sasaki T, Tanishima T, Asano T, Mayanagi Y, Sano K: Significance of lipid peroxidation in the genesis of chronic vasospasm following rupture of an intracranial aneurysm. *Acta Neurochir (Wein)* Suppl 28, vol 2:536-540, 1979.

154 Sasaki T, Wakai S, Asano T, Watanbe T, Kirino T, Sano K: The effect of a lipid hydroperoxide of arachidonic acid on the canine basilar artery. An experimental study on cerebral vasospasm. *J Neurosurg* 54:357-365, 1981.

155 Sasaki T, Wakai S, Asano T, Takakura K, Sano K: Prevention of cerebral vasospasm after SAH with a thromboxane synthetase inhibiter OKY-1581. *J Neurosurg* 57:74-82, 1982.

156 Sato M, Nagao T, Yamaguchi I, Nakajima H, Kiyomoto A: Pharmacological studies on a new 1, 5 benzothiazepine derivative (CRD-401). I. Cardiovascular actions. *Arzneim . Forsch* 21:1338-1343, 1971.

157 Saveland H, Ljunggren B, Brandt L, Messeter K: Delayed ischemic deterioration in patients with early aneurysm operation and intravenous nimodipine. *Neurosurgery* 18:146-150, 1986.

158 Schneck SA, Krichoff II: Intracranial aneurysm rupture, vasospasm and infarction. *Arch Neurol* 11:668-680, 1964.

159 Shigeno T: Norepinephrine in cerebrospinal fluid of patients with cerebral vasospasm. *J Neurosurg* 56:344-349, 1982.

160 Shimizu K, Ohta T, Toda N: Evidence for greater susceptibility of isolated dog cerebral arteries to Ca antagonists than peripheral arteries. *Stroke* 11:261-266, 1980.

161 Silver AJ, Pederson Jr ME, Ganti SR, Hilal SK, Michelson WJ: CT of subarachnoid hemorrhage due to ruptured aneurysm. *AJNR* 2:13-22, 1981.

162 Silver JP, Stull JT: The role of calcium in the contraction of vascular smooth muscle, in: Flaim SF, Zelis R (eds): *Calcium Channel Blockers: Mechanism of Action and Clinical Applications*. Baltimore: Urban & Schwarzenberg, 1982, pp. 37-51.

163 Smith RR: Pathophysiology and Clinical Evaluation of Subarachnoid Hemorrhage, in: Youmans JR (ed): *Neurological Surgery. Developmental and Acquired Anomalies: Vascular Disease*. Toronto: Saunders, 1982, Vol. 3, pp. 1627-1644.

164 Sonobe M, Suzuki J: Vasospasmogenic substance produced following subarachnoid hemorrhage, and its fate. *Acta Neurochir (Wein)* 44:97-106, 1978.

165 Somlyo AP, Somlyo AV, Shuman H, Garfield RE: Calcium compartments in vascular smooth muscle, in: Betz E (ed): *Ionic Actions on Vascular Smooth Muscle*. New York: Springer, 1976, pp. 17-20.

166 Stephens NL, Kroeger EA: Calcium sequestration and relaxation of vascular smooth muscle, in: Varhoutte PM, Leusen I (eds): *Vasodilitation*. New York: Raven Press, 1981, pp. 367-380.

167 Sundt TM, Grant WC, Garcia JM: Restoration of middle cerebral artery flow in experimental infarction. *J Neurosurg* 31:311-322, 1969.

168 Sundt TM, Walz AG, Sayre GP: Experimental cerebral infarction: Modification by treatment with hemodiluting, hemoconcentrating and dehydrating agents. *J Neurosurg* 25:46-56, 1966.

169 Sundt TM Jr., Whisnant JP: Subarachnoid hemorrhage from intracranial aneurysms. Surgical management and natural history of disease. *N Eng J Med* 299:116-122, 1978.

170 Suwanwela C, Suwanwela N: Intracranial arterial narrowing and spasm in acute head injury. *J Neurosurg* 36:314-323, 1972.

171 Suzuki J, Komatsu S, Sato T, Sakurai Y: Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. *Acta Neurochir (Wein)* 55:63-70, 1980.

172 Suzuki J, Onuma T, Yoshimoto T: Results of early operations on cerebral aneurysms. *Surg Neurol* 11:407-412, 1979.

173 Suzuki J, Yoshimoto T: Early operation for the ruptured intracranial aneurysm - especially the cases operated within 48 hours after the last subarachnoid hemorrhage. *Neurol Surg (Tokyo)* 4:135-141, 1976.

174 Svendgaard NA, Edvinsson L, Owman C, Sahlin Ch: Increased sensitivity of the basilar artery to norepinephrine 5-hydrocytryptamine following experimental subarachnoid hemorrhage. *Surg Neurol* 8:191-195, 1977.

175 Tagari PC, Kaye AH, Teddy PJ, Schachter M, Adams CB, Boullin DJ: The 5-hydroxytryptamine antagonists ketanserin inhibits the vasoconstriction activity of per-operative CSF from subarachnoid hemorrhage patients on isolated tissues. *J Neurol Neurosurg Psychiatry* 46:214-220, 1983.

176 Tai HH, Tai CL, Lee N: Selective inhibition of thromboxane synthetase by pyridine and its derivative. *Arch Biochem Biophys* 203:758-763, 1980.

177 Takagi T, Fukuoka H, Nagai H, Hotta K: Basic study of cerebral vasospasm: Ca^{2+} movement during contraction-relaxation of isolated basilar arteries. *Neurol Med Chir (Tokyo)* 19:45-52, 1979.

178 Takagi T, Satake N, Shibata S: The inhibitory action of FR34235 (a new Ca^{2+} entry blocker) as compared to nimodipine and nifedipine on the contractile response to norepinephrine, potassium and 5-hydroxytryptamine in rabbit basilar artery. *Eur J Pharmacol* 90:297-299, 1983.

179 Takahashi S, Sonobe M, Nagamine Y: Early operations for ruptured intracranial aneurysms. Comparative study with computed tomography. *Acta Neurochir (Wein)* 57:23-31, 1981.

180 Tanabe Y, Sakata K, Yamada H, Ito T, Takeda M: Cerebral vasospasm and ultrastructural changes in cerebral arterial wall: An experimental study. *J Neurosurg* 49:229-238, 1978.

181 Tanaka K, Gotoh F, Muramatsu F, Fukuuchi Y, Okayasu H, Suzuki N, Kobari M: Effect of nimodipine, a calcium antagonist, on cerebral vasospasm after subarachnoid hemorrhage in cats. *Arzeimittelforsch* 32:1529-1534, 1982.

182 Taneda M: Effect of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. *J Neurosurg* 57:622-628, 1982.

183 Taneda M, Wakayama A, Ozaki K, Kataoka K, Hayakawa T, Mogami H: Biphasic occurrence of delayed ischaemia after early aneurysm surgery. Case Report. *J Neurosurg* 58:440-442, 1983.

184 Tani E, Yamagata S, Ito Y: Intracellular granules and vesicles in prolonged cerebral vasospasm. *J Neurosurg* 48:179-189, 1978.

185 Tanishima T: Cerebral vasospasm: Contractile activity of hemoglobin in isolated canine basilar arteries. *J Neurosurg* 53:787-793, 1980.

186 Toda N, Ozaki T, Ohta T: Cerebrovascular sensitivity to vasocontracting agents induced by subarachnoid hemorrhage and vasospasm in dogs. *J Neurosurg* 46:296-303, 1977.

187 Toda N, Shimizu K, Ohta T: Mechanism of cerebral arterial contraction induced by blood constituents. *J Neurosurg* 53:312-322, 1980.

188 Towart R: The pathophysiology of cerebral vasospasm, and pharmacological approaches to its management. *Acta Neurochir (Wein)* 63:253-258, 1982.

189 Towart R, Kazda S: The cellular mechanism of action of nimodipine (Bay e 9736), a new calcium antagonist. *Br J Pharmacol* 67:409P-410P, 1976.

190 Towart R, Perzborn E: Nimodipine inhibits carbocyclic thromboxane-induced contractions of cerebral arteries. *Eur J Pharmacol* 69:213-215, 1981.

191 Uski T, Hogestatt E, Edvinsson L, Brandt L, Ljunggren B: Effects of prostanoids on pial vessels. *J Cereb Blood Flow Metab* 1(Suppl 1):S405-406, 1981.

192 Van Breeman C, Aaronson PI, Cauvin CA, Loutzenhiser RD, Mangel AW, Saida K: The calcium cycle in arterial smooth muscle, in: Flaim SF, Zelis R (eds): *Calcium Channel Blockers: Mechanisms of Action and Clinical Applications*. Baltimore: Urban & Schwarzenberg, 1982, pp. 53-63.

193 Vanhoutte PM: Calcium-entry blockers and vascular smooth muscle. *Circulation 65* (Suppl 1):I11-I19, 1982.

194 Varsos V, Liszczak TM, Han DH, Kistler P, Vielma J, Black PM, Heros RC, Zervas NT: Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine or papaverine in a "two hemorrhage" canine model. *J Neurosurg* 58:11-17, 1983.

195 Voldby B, Engbaek F, Enevoldsen EM: CSF serotonin concentration and cerebral arterial spasm in patients with ruptured intracranial aneurysm. *Stroke* 13:184-189, 1982.

196 Von Holst H, Granstroum E, Hammarstrom S, Samuelsson B, Steiner L: Effect of leucotrienes C_4, D_4, prostacyclin and thromboxane A_2 on isolated human cerebral arteries. *Acta Neurochir (Wein)* 62:177-185, 1982.

197 Walker V, Pickard JD, Smythe P, Eastwood S, Perry S: Effects of subarachnoid hemorrhage on intracranial prostaglandins. *J Neurol Neurosurg Psychiatry* 46:119-125, 1983.

198 Walter P, Neil-Dwyer G, Parsons V: The effect of phentolamine and propranalol in subarachnoid hemorrhage, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 584-588.

199 Weir B: Calcium antagonists, cerebral ischemia and vasospasm. *Can J Neurol Sci* 11:239-246, 1984.

200 Weir BK: Pathophysiology of vasospasm. *Int Anaes Clin* 20:39-43, 1982.

201 Weir BKA: The effect of vasospasm on morbidity and mortality after subarachnoid hemorrhage from ruptured aneurysms, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 385-393.

202 Weir BKA: The relationship between arterial spasm and neurological deficits, in: Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp. 45-52.

203 Weir B, Aronyk K: Management mortality and the timing of surgery for supratentorial aneurysms. *J Neurosurg* 54:146-150, 1981.

204 Weir B, Grace M, Hansen J, Rothberg C: Time course of vasospasm in man. *J Neurosurg* 48:173-178, 1978.

205 Weiss GB: Calcium release and accumulation in vascular smooth muscle, in: Flaim SF, Zelis R (eds): *Calcium Channel Blockers: Mechanisms of Action and Clinical Applications*. Baltimore: Urban & Schwarzenberg, 1982, pp. 65-75.

206 Weiss GB: Multiple Ca^{2+} sites and channels provide a basis for alterations in Ca^{2+} mobilization and vascular contractility, in: Vanhoutte PM, Leusen I (eds): *Vasodilitation*. New York: Raven Press, 1981, pp. 307-310.

207 Wellum GR, Irvine Jr TW, Zervas NT: Cerebral vasoactivity of heme proteins *in vitro*. Some mechanistic considerations. *J Neurosurg* 56:777-783, 1982.

208 Wheelock B, Weir B, Watts R, Mohr G, Khan M, Hunter M, Fewer D, Ferguson G, Durity F, Cochrane D, Benoit B: Timing of surgery for intracerebral hematomas due to aneurysm rupture. *J Neurosurg* 58:476-481, 1983.

209 White RP: Multiplex origins of vasoconstriction, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 50-56.

210 White RP, Cunningham MP, Robertson JT: Effect of the calcium antagonist nimodipine on contractile responses of isolated canine basilar arteries induced by serotonin, prostaglandin F_{2a}, thrombin, and whole blood. *Neurosurgery* 10:344-348, 1982.

211 White RP, Robertson JT: Comparison of piroxicam, meclofenamate, ibuprofen, aspirin and prostacyclin efficacy in a chronic model of cerebral vasospasm. *Neurosurgery* 12:40-46, 1983.

212 Wilkins RH: Attempted prevention or treatment of intracranial arterial spasm: A survey, in: Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams & Wilkins, 1980, pp. 542-555.

213 Wilkins RH, Odom GL: Intracranial arterial spasm associated with craniocerebral trauma. *J Neurosurg* 32:626-633, 1970.

214 Wise G, Sutter R, Burkholder J: The treatment of brain ischemia with vasopressor drugs. *Stroke* 3:135-140, 1972.

215 Yamamoto I, Hara M, Ogura K, Suzuki Y, Nakani T, Kageyama N: Early operation for ruptured intracranial aneurysms: Comparative study with computed tomography. *Neurosurgery* 12:169-174, 1983.

216 Yashon D, Brown RJ, Hunt WE: Vasoactive properties of prostaglandin compounds on the *in vitro* human basilar artery. *Surg Neurol* 8:111-115, 1977.

217 Yoshida Y, Ueki S, Takahashi A, Takagi H, Torigoe H, Kudo S: Intrathecal irrigation with urokinase in ruptured cerebral aneurysm cases: Basic study and Clinical application. *Neurol Med Chir (Tokyo)* 25:989-997, 1985.

218 Zabramski J, Spetzler RF, Bonstelle C: Chronic cerebral vasospasm: Effect of calcium antagonists. *Neurosurgery* 18:129-135, 1986.

219 Zervas NT, Kistler JP, Ploetz J: Effect of reserpine and kanamycin on post-operative delayed ischemic deficits in patients with subarachnoid hemorrhage after aneurysm rupture, in: Wilkins RH (ed): *Cerebral Arterial Spasm.* Baltimore: Williams & Wilkins, 1980, pp. 514-517.

220 Zervas NT, Liszczak TM, Mayberg MR, Black PM: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analagous to systemic vasa vasorum. *J Neurosurg* 56:475-481, 1982.

PUBLICATIONS

Nosko M, Weir B, Krueger C, Cook D, Norris S, Overton T, Boisvert D: Nimodipine and chronic vasospasm in monkeys. Part 1. Clinical and radiological findings. *Neurosurgery* 16:129-136, 1985.

Krueger C, Weir B, Nosko M, Cook D: Nimodipine and chronic vasospasm in monkeys. Part 2. Pharmacological studies of vessels in spasm. *Neurosurgery* 16:137-140, 1985.

Weir BKA, Espinosa F, Nosko M, Norris S, Cook DA, Krueger CA: Development of a primate model of chronic vasospasm and delayed ischemic deficit - attempts at modification using nimodipine. Proceedings of the First International Nimotop Symposium, in: Betz E, Deck K, Hoffmeister F (eds): *Nimodipine, Pharmacological and Clinical Properties*. Munich: F.K. Schattauer, 1985, pp 375-377.

Nosko M, Norris SL, Weir B, King EG, Grace M: Nimodipine and chronic vasospasm in monkeys. Part 3. Cardiopulmonary effects. *Neurosurgery* 18:261-265, 1986.

Norris SL, Nosko M, Weir B, King EG, Grace M: Acute cardiopulmonary effects of subarachnoid hemorrhage in monkeys. *Crit Care Med* 14:491-494, 1986.

Nosko M, Krueger CA, Weir BKA, Cook DA: Effects of nimodipine on *in vitro* contractility of cerebral arteries of dog, monkey and man. *J Neurosurg* (In Press).

Hara H, Nosko M, Weir B: Cerebral perivascular nerves in subarachnoid hemorrhage: A histochemical and immunohistochemical study. *J Neurosurg* (In Press).

Nosko M, Weir B, Lunt A, Grace M, Allen P, Meilke B: Clot removal at 24 hours prevents chronic vasospasm after subarachnoid hemorrhage in the primate model. Submitted for publication.

Nosko M, Schulz R, Weir B, Cook DA, Grace M: Basal cerebral arterial levels of prostacyclin and thromboxane A_2 after subarachnoid hemorrhage in monkeys. Submitted for publication.

Nosko M, Weir B, Overton T: Cerebral blood flow and cardiopulmonary effects of intravenous nimodipine in the monkey. Submitted for publication.